THE HARVEY SOCIETY

THE HARVEY LECTURI

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THE HARVEY SOCIETY
OF NEW YORK

1915-1916

BY

PROF. CH. WARDELL STILES
PROF. A. J. CARLSON
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PROF. FLORENCE R. SABIN
DR. DONALD D. VAN SLYKE
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SERIES XI

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PREFACE

The Harvey Society presents to the public in this volume the papers comprising the eleventh series of lectures. The list of contributors differs from those of previous years in that no foreign country is represented. Heretofore, it has been the custom of the Society to invite as lecturers men of science without regard to country or nationality. However, in planning the program for the past season it was not deemed expedient on account of the existing war, which has made international communication slow and difficult, to extend invitations to any one outside our own country. While this decision necessarily limited the number of those available as lecturers, the Society has reason to congratulate itself on the result; for this series of lectures, as judged by attendance and interest manifested, was probably the most successful in the Society's history. We take this opportunity to extend the thanks of the members of the Harvey Society to the speakers of the year whose splendid and generous service has made this success possible.

Following the custom of former years of making acknowledgment to journals in which any of the lectures have already been published, we wish to express our indebtedness to the American Journal of Medical Sciences for the re-publication of the papers by Professor Longcope and Dr. Du Bois.

Robert A. Lambert, *Secretary.*

November, 1916.

THE HARVEY SOCIETY

A SOCIETY FOR THE DIFFUSION OF KNOWLEDGE OF THE MEDICAL SCIENCES

CONSTITUTION

I.

This Society shall be named the Harvey Society.

II.

The object of this Society shall be the diffusion of scientific knowledge in selected chapters in anatomy, physiology, pathology, bacteriology, pharmacology, and physiological and pathological chemistry, through the medium of public lectures by men who are workers in the subjects presented.

III.

The members of the Society shall constitute three classes: Active, Associate, and Honorary members. Active members shall be laboratory workers in the medical or biological sciences, residing in the City of New York, who have personally contributed to the advancement of these sciences. Associate members shall be meritorious physicians who are in sympathy with the objects of the Society, residing in the City of New York. Members who leave New York to reside elsewhere may retain their membership. Honorary members shall be those who have delivered lectures before the Society and who are neither active nor associate members. Associate and honorary members shall not be eligible to office, nor shall they be entitled to a vote.

Members shall be elected by ballot. They shall be nominated to the Executive Committee and the names of the nominees shall accompany the notice of the meeting at which the vote for their election will be taken.

CONSTITUTION

IV.

The management of the Society shall be vested in an executive committee, to consist of a President, a Vice-President, a Secretary, a Treasurer, and three other members, these officers to be elected by ballot at each annual meeting of the Society to serve one year.

V.

The Annual meeting of the Society shall be held soon after the concluding lecture of the course given during the year, at a time and place to be determined by the Executive Committee. Special meetings may be held at such times and places as the Executive Committee may determine. At all the meetings *ten* members shall constitute a quorum.

VI.

Changes in the Constitution may be made at any meeting of the Society by a majority vote of those present after previous notification of the members in writing.

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RECENT STUDIES ON SCHOOL CHILDREN, WITH SPECIAL REFERENCE TO HOOK-WORM DISEASE AND SANITATION *

PROF. CH. WARDELL STILES

U. S. Public Health Service

CONSERVATION" and "efficiency" are two of the most popular catchwords of the day. To many persons, they seem to represent new lines of thought. When analyzed, however, they are seen to represent in reality two of the most important elements contained in the old-fashioned word "economy." This word economy is not a popular one, and if one takes it as a text he is not very likely to have an attentive audience, for the word immediately brings up thoughts of sacrifices, notwithstanding the fact that the first essential of economy is to prevent unnecessary "waste" rather than to do without something that is valuable, necessary, or desirable.

The best and most practical definition or description of economy I have ever heard is that it consists in spending the greatest amount of money that will give the greatest possible returns.

This evening I invite your attention to certain phases of economy as applied to the health and advancement of school children, but in order to avoid misunderstanding permit me to emphasize the point that my text involves not a restriction in expending money or care, but rather an increased expenditure of both in order to bring about the fundamental elements of economy, namely, prevention of waste and promotion of conservation and efficiency.

To the popular mind, it is the "epidemic" of unusual disease that is something to be dreaded, and the average person rarely

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* Delivered October 16, 1915.

stops to think that the aggregate of little losses, here and there, may present a grand total which far exceeds that from some of the much dreaded epidemics. How few persons recall, for instance, that in this country the annual loss in human life from tuberculosis alone is greater than that we have suffered from yellow fever during more than a century. Yet the public excitement caused by one case of yellow fever is much greater than that caused by more than a hundred thousand cases of tuberculosis. In the case of yellow fever we have the sudden appearance of an unusual disease, while in the hundred thousand cases of tuberculosis we have a daily drain, a daily waste, that results in a total of much greater sorrow and inefficiency.

To take a more familiar illustration, compare in your mind the public comment produced in a school district by the appearance of an epidemic of measles, chicken-pox, or whooping-cough, with the apathy usually attending the presence of adenoids, enlarged and infected tonsils, poor eyesight, poor teeth, or a heavy endemic infection of hookworm disease.

Without multiplying comparisons further, I would present as my thesis this evening the point that economy in public school administration (or, if you prefer, conservation and efficiency among our school children) calls for a greater expenditure of care and money in order to prevent the constant little daily drains on child-life that are due to such factors as adenoids, enlarged tonsils, defective eyesight, poor teeth, and to poor sanitation at schools and at homes, insanitation that results in such common infections as dysentery, diarrhœa, typhoid fever, hookworm disease, and other excreta-borne maladies.

One of the earliest recollections of my childhood is the expression, "If you take care of your pennies, the dollars will take care of themselves." The same general principle holds in public health, namely, if we are careful about little things in conserving health and life, many of the larger problems will automatically take care of themselves.

In recent years, I have been especially interested in certain phases of child-life. Some of my friends have accused me of being somewhat interested in hookworms, but as a matter of fact

the hookworm, from my point of view, is and always has been only an incident, a stage-setting or base of supplies, so to speak, in attacking a much broader problem of child-life and efficiency, as influenced by sanitation.

From the cold-blooded stand-point of zoölogy, the common hookworm of this country is a diœcious nematode belonging to the superfamily *Strongyloidea,* family *Strongylidæ,* sub-family *Uncinariinæ,* genus *Necator,* species *Necator americanus,* with this, that, and the other technical details that are of considerable interest to a few specialists (possibly twenty in the entire world) in helminthology, and of little interest to the public in general.

From a warm-blooded human stand-point, however, the hookworm is a factor that makes for suffering, sickness, inefficiency, and retardation. What, for instance, can a teacher expect to accomplish with groups of children such as you see in the picture before you? It is this, not the zoölogical view-point, that especially appeals to me. It is the hookworm child and its mother, rather than the hookworms that occupy my thoughts, and this only as representing an example of a large class of conditions that can and should be changed for the better.

While studying hookworm infection in school children, I have frequently been impressed by the fact that in general the sanitation of the home is a practical working index to the physical condition of rural children, and in order to reduce these results of general observation to concrete examples, and especially in order to see whether the differences apply also to urban children, I have recently studied the school children of a certain county, which we may call X, located in the sandy area of the South and in which the whites happen to outnumber the negroes.

Because of the uncertainty as to exact age, a considerable number of negro pupils and not an inconsiderable number of the white students had to be eliminated from all summaries in which age-groups must be considered. This may appear strange to you, for one is accustomed to view a knowledge of one's birthday and exact age as self-understood, and a lack of that knowledge as a lack of intellectual development. Has it, however, occurred to you that the custom of giving birthday parties and birthday

presents is an important factor in impressing upon one's memory his birthday and birthyear, and that as the average per capita wealth decreases so that there are many families that cannot afford birthday presents, a knowledge of one's birthday is less essential to happiness and less likely, therefore, to obtain? One evening I had 14 girls, varying from about 7 to 15 years of age, in a railroad hospital car; it was near Christmas time, and I asked the children about their dolls; 8 out of 14 of these girls stated that they had never owned a doll. Birthdays meant nothing to these children, and Christmas meant little more. Lack of knowledge regarding one's age is in these cases not so much a question of lack of intellect or a retardation in mental age, but a question of the family pocket-book which happens to be so thin that even a doll cannot be purchased for a 7-year-old girl! So there is nothing strange in the fact that some of my groups are reduced in number of members by the circumstance, for instance, that not a few of my children or their parents had little reason to remember ages and birthdays. I know in fact cases where children could not attend school regularly simply because they did not have sufficient clothing to comply respectably with ordinary police regulations.

My age-groups consist of quarter-year groups which are then summarized into total-year groups. For instance, all 10-year-old children fall into the groups 10 years flat, 10.25, 10.50, and 10.75 years old; and they are summarized into a 10-year-old group.

The children are also classified by sex, and further by sanitary groups, as follows: Group S includes the children from homes provided with sewer connection, but without privy; Group P includes the children known to be from homes provided with privies; and Group U includes children from homes of unknown sanitation. It is self-understood that with this extensive subdivision, some of the sex-quarter-year-sanitary groups contain very few individuals, but with this point in mind it is legitimate to inquire whether the total-year sanitary groups show any particular tendencies in any direction. In general, do the children of Group S (with sewer sanitation) and those of Group P (with privy sanitation) show any differences, and if so what are these differences?

INTESTINAL PARASITES

One of our tests was a microscopic examination to determine what intestinal parasites were present. The results, though not unforeseen, are instructive and to my mind important:

There was a total of 3594 city pupils, of whom 2248 were white and 1346 negro; 32 per cent. of the whites and 38 per cent. of the negroes furnished specimens for microscopic examination; thus in this test the negroes co-operated somewhat better than the whites; 49 per cent. of the negroes examined, and 37 per cent. of the whites examined showed infection with one kind or another of intestinal parasite. This difference is largely due to the fact that in general the negroes live under poorer sanitary conditions than do the whites, for 76 per cent. of the total negroes, as compared with 20 per cent. of the total whites, belong to Group P.

In considering the sanitary groups, we find that in Group P 50 per cent. of the white children and 50 per cent. of the negro pupils show intestinal parasites, as compared with Group S in which 34 per cent. of the whites and 41 per cent. of the negroes show infection. It makes no difference whether we consider the totals, or the two races, or the two sexes, the general conclusion is the same, namely, that intestinal infections are more common in children who live at homes with poorer sanitation than among those who live at homes with better sanitation.

Intestinal infections are more common among white boys than among white girls, but negro boys show approximately the identical percentages of these parasites as do negro girls. If, however, the figures are analyzed closely the conclusion seems justified that some of the infections take place away from the home, and this seems to hold especially for the white boys.

Coprophagia.—The parasites in question naturally fall into two biological categories, namely, 6 species that may be contracted in one way and in one way only, by actually swallowing, doubtless accidentally, minute amounts of human excrement, and two species not necessarily contracted by this unconscious coprophagia. For instance, if we find *Endamœba, Lamblia, Trichomonas,* eelworms (*Ascaris*), pinworms (*Oxyuris*), or whipworms (*Trichuris*), we have positive, absolute, undeniable proof that that

person has actually swallowed germs that have come from the excreta of some other person, a proof that holds equally well when we find that a person is suffering from typhoid fever, Asiatic cholera, and certain forms of diarrhœa and dysentery. This proof is not present, however, in case we find tapeworms or hookworms.

If now we analyze our statistics from this point of view, we have positive proof that 28 per cent. of the white children and 48 per cent. of the negro pupils had actually swallowed human excrement. This means that it is merely a matter of chance that about one-fourth of the white children and about one-half of the negro children did not present typhoid, or a history of recent typhoid.

Comparing the two races, it is seen that the negro pupil runs a greater chance of unconscious coprophagia than does the white, and comparing the two sexes of each race we find that the white boys run a greater chance of coprophagia than do the white girls (namely, 34 to 20), while in the negro the chances are more equal (namely, about 47 in the boys to 49 in the girls). Comparing next the sanitary groups, we find that children of Group P show a greater amount of coprophagia than do children of Group S. Thus, in white girls the chances are 24 to 19, in white boys 40 to 33, in total whites 32 to 27, in negro girls 50 to 44, in negro boys 49 to 33, and in total negroes 49 to 41.

These figures fully justify us in expecting that children of Group P will have a greater proportion of absenteeism due to such diseases as typhoid, diarrhœa, and dysentery, than will children of Group S, and that, consequently, children who live under poorer sanitation will show a greater retardation in school advancement due directly to absenteeism caused by these diseases than will be found among children who live under better sanitation.

Making a broad application of this conclusion, it seems evident that the home sanitation under which children live has a direct influence upon the administration of school funds. That is to say, the school authorities cannot possibly expect to have the same

total or average educational returns per $1000 expended upon teaching 1000 children who live under poorer sanitary conditions that they can expect to have in the case of 1000 children who live under better sanitary conditions. To put it more bluntly, it costs more per capita to obtain a given amount of education among poorer children than it does among children from families in better financial circumstances, and the conclusion is therefore justified that an increase in the amount of sanitary supervision and improvement of the sanitation under which the poorer families live means an actual economy in the public school funds.

To the professional sanitarian and to the professional educator, this conclusion is, or should be, almost axiomatic. To the taxpayer, however, it is less evident. In the statistics presented on the subject of unconscious coprophagia we have, I believe, an argument which should appeal to parents in general, and which has already been an important factor among the citizens of the County of X in arousing their determination to improve sanitary conditions in general.

Another conclusion follows from these studies, namely, that the influence of the sanitation at a given home is a matter which involves not only the members of that particular home but also the community in general, for our experiments clearly show that a given backyard has an influence which may radiate in all directions of the compass, and which may, therefore, influence the neighbors, for we have actually demonstrated by experiment that some of the parasites in question can be carried by flies and that these flies may therefore spread the excrement at one home to the food of the neighbors.

Turning next to the second category of parasites, namely, those not necessarily contracted by coprophagia, our examinations show that among the white children hookworm infection was about three times as common in children of Group P as in pupils of Group S. Since, now, hookworm infection has a direct effect in inhibiting both mental and physical development, we must naturally expect greater retardation from this cause among children of Group P than among children of Group S.

SCHOOL-GRADE ADVANCEMENT

Let us next examine the actual school-grade advancement of the white pupils. My statistics show this point for 2166 students, 1062 boys, 1104 girls.

From the data at hand, it seems clear that the girls made 80 per cent., the boys 78 per cent. of the school grades to which they were theoretically entitled, as estimated on basis of their exact age. If we compare the sanitary groups, the point develops that the girls of Group S made 84 per cent. as compared with 72 per cent. attained by the girls of Group P, while the boys of Group S made 81 per cent. as compared with 68 per cent. attained by Group P.

If we study the cases of infection with *Endamœba coli* or with *Lamblia,* no evidence is obtained that these two parasites had any measurable effect in the retardation noted. Our data for *Trichomonas,* pinworms, whipworms, and tapeworms are not sufficient to warrant deductions. But although the 83 hookworm infections were, in general, not severe, they show a total extra retardation of 17.56 grades or school years, as compared with their respective groups, thus giving an average of nearly 1/4 grade (0.23 grade) per child. When we consider the relatively light degree of infection found, and when we compare these figures with our general experience in rural schools, I do not hesitate to interpret this retardation of 17.56 years as due in large degree to the hookworm infection.

The 58 pupils infected with eelworms showed a total extra retardation of 3.45 years as compared with the average of their respective groups. This average extra retardation of 0.07 grade per pupil seems negligible when considered alone, but it represents one of the little drains that could easily be avoided.

TOBACCO HABITS

If we study the tobacco habits and consider only those cases for which these habits are admitted either by the children, or for them by their parents, the point develops that smoking was admitted by a greater percentage of children in Group S and

chewing was admitted by a greater percentage of children in Group P. This result is in harmony with my studies of rural school children in another county, where I found that smoking increased and chewing decreased coincident with the improved sanitary conditions found at the homes.

HEIGHTS AND WEIGHTS

For standing height, sitting height, and weight of the white pupils, data are present as follows:

In general the sitting height is a little more than one-half of the standing height, but in girls from 13 to 17 years inclusive, it is considerably more than one-half of the standing height.

The children show two rather striking interruptions in growth. At 11, there is a rather striking decrease of the increase in the standing height, sitting height, and weight of the boys, and a less marked decrease of the increase in the sitting height of the girls.

At 14, there is a sudden and very pronounced decrease of the increase in the standing height, the sitting height, and the weight of the girls. In this connection it is rather suggestive that these girls average their first menstruation at 13.2 years of age. The change in growth of the boys is much less striking, but they show a marked interruption in the increase in weight. In general, the growth of boys from 13 to 17 is far in excess of that of the girls, and this is especially marked at 17 years.

Of the total-year periods (12 for boys, 12 for girls), Group S excelled in standing height in 17 periods, Group P in 7 periods; in sitting height, Group S excelled in 13 periods, Group P in 11 periods; in weight, Group S excelled in 15 periods, Group P in 9 periods. Thus, in the total of 72 units, Group S excelled in 45 units (62.5 per cent.) and Group P in 27 units (37.5 per cent.).

No evidence was obtained that infection with *Ascaris, Lamblia,* or *Endamœba* was a measurable factor in retarding growth, but it may be noted that the infections with *Ascaris* were light. The data for whipworms are too limited for deduction.

In children showing infection (light or rather light cases) with hookworms, the evidence is not striking but it summarizes as follows:

	Below average	Above average
Standing Height	30	21
Sitting Height	35	25
Weight	26	22
	—	—
	91	68

Thus, in final score the hookworm cases were below average in 91 markings and above the average in 68 markings. The conclusion appears, therefore, to be justified that even in the light cases with which we were dealing, the infection had an appreciable effect on heights and weights.

LUNG CAPACITY

Our white boys from 6 to 13 years old inclusive showed an average from 100 to 200 c.c. greater lung capacity than the girls of the same age. From 14 to 17 years they had progressively from about 300 to about 1100 c.c. greater lung capacity than the corresponding girls. In the case of both the boys and the girls, the children from homes with better sanitation had a tendency (15 to 9) to greater lung capacity than the children from homes with poorer sanitation (9 to 15). Our studies did not show that infection with hookworms, *Ascaris, Lamblia,* or *Endamœba coli* had any noticeable effect upon the spirometer tests.

BLOOD EXAMINATIONS

Data on complete blood examinations are present for 574 white pupils 295 boys, 279 girls. In total red blood-cells Group S excels in 15 total-year periods (6 for boys, 9 for girls) and Group P excels in 7 total-year periods (5 boys, 2 girls), while for 2 periods no comparison could be made. It is self-understood that some of the groups are reduced to a very small number of individuals, but if one objects strenuously to this point we may compare the average of grand totals for Group S with those for

Group P. This comparison shows that the 234 boys of Group S average 4,633,000 as compared with an average of 4,591,000 for the 51 boys of Group P, while the 200 girls of Group S average 4,752,000 as compared with an average of 4,498,000 for the 74 girls of Group P.

In hæmoglobin, Group S excels in 13 total-year periods (7 for boys, 6 for girls) and Group P excels for 9 periods (4 for boys, 5 for girls). Taking the average for all children of these groups, the 234 boys of Group S averaged 86.7 per cent. as compared with an average of 84.4 per cent. for 51 boys of Group P, and 200 girls of Group S averaged 87.7 per cent. as compared with an average of 87.5 per cent. for girls of Group P.

The leucocytes excelled in 13 total-year periods (7 for boys, 6 for girls) in Group P as compared with 9 total-year periods (4 for boys, 5 for girls) in Group P. For the 234 boys of Group S the leucocytes averaged 7860 as compared with an average of 8687 for 51 boys of Group P, and for the 200 girls of Group S they averaged 7731 as compared with an average of 7771 for 74 girls of Group P. As a high leucocyte count is indicative of infection of some kind, it is seen that the comparisons made on basis of total-year periods indicate a greater average of infections in Group P than in Group S.

MEMORY SPAN IN NUMBERS

One of the various mental tests used was the common test for the memory span, a test that is so frequently utilized in psychological and psychiatric studies. Data were obtained for 1581 urban white pupils, 748 boys, 833 girls, from 6 years flat to 17.75 years old, inclusive. Summarized, our results show that in the locality in question, children of 6 flat to 7.75 years inclusive can reasonably be expected to have a memory span of 5 numbers, children from 8 flat to 13.75 inclusive can be expected to have a memory span of 6 numbers, and children of 14 flat to 17.75 inclusive can be expected to have a memory span of 7 numbers. These figures are based upon the averages of the total-year groups.

If the boys are compared with the girls, the conclusion seems justified that the differences are not of sufficient constancy to

justify the conclusion that either sex excels. If the sanitary groups are compared the conclusion seems justified that the comparison is more favorable to Group S in the proportion of 14 to 10 or 7 to 5, for 7 total-year male groups and 7 total-year female groups of Group S excelled as compared with 5 total-year male groups and 5 total-year female groups of Group P.

A further analysis of the statistics shows that in the 55 infections with *Endamœba coli,* in the 67 infections with *Lamblia,* and in the 38 infections with eelworms, we are not justified in concluding that these parasites resulted in an average decreased memory span; our 52 cases of hookworm infection showed an average loss of 0.08 (boys 0.15 loss, girls 0.06 gain) when compared with the average of their respective groups. As already stated the infections were in general rather light.

Knox Cube Test.—For another mental test, known as the Knox Cube Test, somewhat similar results were obtained.

Our results show that the urban white children of 6 flat to 7.75 years, inclusive, in the region in question may be reasonably expected to reach C in this test; while children from 8 flat to 17.75 years inclusive may be reasonably expected to reach D. These figures are based upon the total-year averages. As a matter of fact, however, 21 out of 61 children of the 6 total-year group attained E and 36 out of 49 of the 17 total-year group attained E. The oldest child to attain A was in the 14.75 year subgroup.

Comparing the total-year sanitary groups, the final score stands 14½ to 9½ in favor of Group S; that is to say, among the boys, Group S excelled in 9 total-year periods as compared with Group P, which excelled in 3 total-year periods, while among the girls Group S excelled in 5 periods, Group P 6 periods, and the two groups were equal in 1 period.

SUMMARY

The statistics thus far presented for the county of X seem to confirm the point that school children who live at homes with better sanitation are ahead of school children from homes with poorer sanitation, for the figures show that in general and on the average the comparison is more favorable to children of

Group S than to those of Group P in the following 14 particulars:

1. Children of Group S show a lower percentage of intestinal parasites in general.
2. They show a lower index of infection with *Lamblia*, eelworms, whipworms, and hookworms, all of which are more or less pathogenic. For the other parasites, except *Endamœba coli* which is not pathogenic, our cases are too few in number to warrant emphasis.
3. They show a lower index of coprophagia, therefore,
4. They are less liable to contract typhoid, diarrhœas, and dysenteries.
5. They show a better growth in standing height.
6. They show a better growth in sitting height.
7. They show a better growth in weight.
8. Their lung capacity is *slightly* better.
9. They average a higher red blood count.
10. They average a higher percentage of hæmoglobin.
11. They average a lower leucocyte count, hence indications are that certain bacterial infections are less frequent or less severe.
12. They make a higher percentage of their theoretical school grades.
13. They average a higher mental span.
14. They make a higher average in the Knox Cube Test.

Doubtless, the question has already arisen in your minds as to how much of the rather consistent differences noted between Group S and Group P is due to the sanitation of the home, and how much is simply coincident with the sanitation but due to other factors, such as heredity, food, economic status in general, educational status of the parents, and a dozen or more other factors that come up in your minds. The question is an eminently fair one, but the answer is difficult to express in percentages.

In my answer to your query, I would make the following points in particular:

First. The spread of all of the parasites mentioned is entirely due to lack of proper sanitation and to no other cause. If excreta

were properly disposed of, both in the direct environment in which these children are living and in the more distant environments that influence their lives, not one of the intestinal parasites would be present.

Second. Under proper public health conditions, such infections as typhoid, dysentery, and diarrhœa would nearly or entirely disappear.

Third. Several of the infections mentioned play rôles which may be greater or less, often unmeasurable, according to circumstances, and if the sanitation is improved, the differences due to these infections would gradually disappear.

Fourth. The insanitation, in being responsible for bacterial, protozoal, and verminous infections mentioned, forms a vicious circle with the general economic conditions, each contributing to the index of the other, and if the sanitation is improved, then the economic condition will improve to the extent that the lowered economic condition may be due to inefficiency, sickness, and death in the families, caused by disease that is spread by insanitation.

Fifth. The increased economic status that can be obtained by eliminating one fundamental cause (namely, insanitation) of its decrease will react with a good percentage of annual interest in enabling the family to combat other causes that decrease its economic status.

Sixth. It would be fundamentally wrong to claim that the question of sanitation alone is the only factor involved, but it is undoubtedly one of the many factors that must be considered and it forms a general working index by which we may actually classify our school children into two groups (S and P) that actually show differences when the children are compared.

Seventh. The insanitation under which many school children are to-day living, not only at their homes but, sad to relate, at the public schools and churches they attend, is the cause of a daily drain upon them which can easily be eliminated, and the elimination of this insanitation is one factor in the economy we should have in mind, namely, the spending of a greater amount of money and care for the sake of the conservation and efficiency of child-life.

MATHEMATICAL ESTIMATE OF DISEASE

The question naturally arises whether it is possible to give an approximate or working estimate in terms of mathematics comparing diseased conditions of children, somewhat similar in nature to the statistical studies of psychologists in their experimental work. I know of no published attempt of this kind in reference to Southern conditions. The attempt to apply to the effects of disease the comparative mathematical methods used in certain collateral fields of work is indeed tempting, and not long ago the opportunity for such an attempt was presented in a study of hookworm children of the County of Z, conducted as a co-operative undertaking between the Rockefeller Sanitary Commission and the U. S. Public Health Service. The details of the mental work will be published by the Rockefeller Foundation and of the medical work by the U. S. Public Health Service.

In these studies, I had under observation about 175 white rural school children, taken at random, except for age, from several near-by rural schools, all located in the same county.

In the final results, 115 children were available for comparison.

First, the children were subjected to a thorough physical schedule study lasting three hours or more per child, including blood counts; then, some of the children were put under medical treatment; after the lapse of time averaging about three months they were re-examined on the same schedule plan; finally, they were divided into 5 groups, A to E; the composite characters of the groups were summarized, and the groups were compared.

That the method has not exact mathematical exactness is self-understood, because not all physical characters have the same value. Nevertheless, the final results compare very favorably with general experience and seem to be justified. The least that can be said of the results is that as a first attempt of this kind, as applied to hookworm disease, the figures are interesting. But since the same chances for error apply to all the children, I feel that the results are more than simply interesting. In fact, I feel that they are about as exact as it is possible, at present, to obtain, and that they must be taken seriously, at least until someone develops a much better method.

Group A contained 23 negative controls, namely, children who, upon microscopic examination in the first test, showed no hookworm infection; Groups B–E showed this infection.

Group B contained 12 positive controls, namely, children whom I did not treat for hookworm disease, while Groups C–E were treated.

Group C contained 36 infected children from whom an average of 40 hookworms per child was collected, and at the third test they showed no evidence of hookworm infection; we may call them the completely cured.

Group D contained 32 infected children from whom an average of 93 hookworms per child was collected, but at third test they still showed some evidence of infection; they were incompletely cured.

Group E contained 12 infected children from whom an average of 100 hookworms per child was collected, but they failed to furnish specimens at the third test, so it is impossible to state whether all their worms were expelled.

Let us now conceive of these 5 groups of children as representing 5 colleges that send representatives to an athletic track meet, and let us accept each of the 186 physical characters considered as representing a separate entry in the contest; then let us apply the point system of athletics to our results, and distribute $10 for each first place made by a group, $8 for each second place, $6 for each third place, $4 for each fourth place, and a consolation prize of $2 for each fifth place. Let us then summarize the prizes for each group and see where they stand.

The chart shows that Group A, consisting of 23 non-infected children, easily won first place in the first test, namely, in the pre-treatment examination, by taking $1485 in prizes.

Group B, consisting of 12 infected children, took third place by winning $1098 in prizes.

Group C, consisting of 36 infected children, took second place by winning $1242.

Group D, consisting of 32 infected children, took fifth place by winning $823 in prizes.

Group E, consisting of 12 infected children, took fourth place by winning $932 in prizes.

These ratings represent the comparative physical efficiency of the 5 groups, as nearly as it was possible to express this in figures, on basis of the first physical study of three hours or more to each child, and they represent the composite work of several examiners.

Recall, now, that Groups C–E were treated and that an average of 40 hookworms was obtained from the children in Group C, 93 from those in Group D, and 100 from those in Group E. After a lapse of time another track-meet or examination takes place and the same 186 entries or physical characters are considered. The summary shows that:

Group C, which was second, now wins first place, with $1366 in prizes; after expelling their hookworms, these 36 children actually went ahead of the 23 children who in the first meet showed no hookworm infection.

Group A, our 23 negative controls, which won first place in the first meet, dropped to second place in the last meet, but carried off $1290 worth of prizes.

Group E, which had fourth place originally, won third place with $1093 worth of prizes, after expelling an average of 100 hookworms.

Group D, the original tail-ender, rose to fourth place, with $984 worth of prizes, as a result of expelling an average of 93 hookworms.

And Group B, our positive controls, 12 hookworm children who won second place in the first meet, but were not treated, dropped to fifth place in the last meet by taking only $847.

These figures for the last meet represent to my mind approximately the relative physical efficiency of the 5 groups of children in the post-treatment examination.

If now we compare the relative improvement made between the first and last examination, it is seen that:

Group D had the greatest outlook for improvement since it stood fifth in the first test; actually it gained first place in improvement by taking $1329 worth of improvement prize money.

Group E had the second best chance potentially, as it originally stood fourth; it actually gained second place in the improvement by winning $1161 in improvement prize mo

Group B had the third best chance, for it stood third in the first test; but the children were not treated and the Group made only fifth place, with $634 in improvement prize money.

Group C had potentially fourth chance, but it made third place, with $1160 in improvement prize money.

Group A had least chance for improvement, potentially, as it took first place in the first test, but it made fourth place in the improvement by gaining $816 in improvement prize money, due to the fact that the hookworm Group B continued to keep its infection.

This presentation is, admittedly, a rather unusual method of presenting a medical subject, but I believe it is thoroughly justified.

Taking the two presentations given to-night, one a comparison of school children who live under better sanitary conditions with school children who live under poorer sanitation, and following that with a study of one of the diseases which owes its spread to insanitation, I submit that I have demonstrated my thesis for the evening, namely, that one of the premises in the economy in administration of public school funds is the sanitation under which the school children live at home and at school. A corollary to this thesis is that an increase in expenditure of care and money in improving this sanitation will result in an actual economy in child-life in the terms of conservation and efficiency.

ON THE NERVOUS CONTROL OF THE HUNGER MECHANISM *

PROF. A. J. CARLSON
University of Chicago

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* Delivered November, 1915.

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THE question of the nervous control of the gastric hunger mechanism embraces several important physiological problems, none of which is as yet completely solved:

First, on the motor side there is the possibility of actual initiation of the gastric hunger contractions through the motor fibres in the vagi nerves by impulses from cerebral as well as lower centres acting on the motor nuclei of the vagi in the medulla. Even if the contractions are not actually caused in this manner, it can be shown that they are in part dependent on a " tonus " influence exerted on the stomach by the vagi nerves. Hence the control of the vagi tonus becomes a question of paramount importance in the physiology and pathology of hunger.

Second, on the afferent or sensory side we must determine central paths of the afferent gastric nerves in order to elucidate the genesis of the conscious hunger sensation, as well as the conscious and sub-conscious reflexes evoked by these afferent impulses. This raises the question of the sensory hunger centre in the cerebrum.

Third, we have also to deal with the very important reflex control of the gastric hunger mechanism as well as of the nervous foci in the medulla, mid-brain, and cerebrum concerned in the conduction of sensory and motor hunger impulses.

And, last, we must consider the automatic or reflex elements in the gastric hunger mechanism itself, independent of all central nervous system control. An understanding of these several factors is of particular importance for the interpretation and the control of the changes in hunger and appetite that we meet in disease.

I. CENTRAL CONTROL OF THE HUNGER MECHANISM

1. *Effect of Removal of the Cerebrum.*—Removal of the cerebral hemispheres in the guinea-pig leads to somewhat increased gastric tonus and hunger contractions (King). In the pigeon this operation does not change the hunger contractions of the empty crop, except that visual and auditory stimuli do not lead to inhibition of these movements in the decerebrated bird (Rogers). In frogs removal of the cerebrum has no effect on hunger contractions

of the stomach (Patterson). We may, therefore, conclude that in so far as the stomach hunger contractions are dependent upon tonus and motor nervous impulses via the vagi nerves, these impulses do not originate in the cerebral hemispheres.

2. *The Gastric Hunger Mechanism during Sleep.*—In man (infants as well as adults) the gastric hunger contractions are at least as frequent and intense during sleep at night as during the waking state. In our five days' starvation experiment continuous records of the stomach were taken during sleep at night. These records show that the author's stomach was in strong tonus and hunger contractions practically half of the time of sleeping. The hunger periods were less frequent during the day when the subject was about his work.

Numerous experiments on dogs show the hunger contractions and the gastric tonus more vigorous and regular when the animal is sleeping than when he is awake and taking notice of things about him. The only apparent exception to this condition so far observed in any species is the rumen of the goat. A few observations on one goat seemed to show that the hunger contractions of the rumen, or first stomach pouch, decreased in intensity when the animal was lying down sleeping. We are not satisfied that this is so until the same result is obtained on a number of ruminants. Possibly the gastric motor part of the vagi nervous apparatus in the ruminating animals is under a more direct control from the cerebrum than in other species.

During sleep there is decreased activity of the central nervous system in general, decreased tonus of the skeletal muscles, decreased tonus of the musculature of the blood-vessels, at least in certain parts of the vascular system, decreased tonus of the urinary bladder, etc.; in short, a lowered activity of all the neuromuscular mechanisms so far investigated. One might have expected that in so far as the tonus of the empty stomach depends on a central influence by way of the vagi, the gastric tonus and hunger contractions should be diminished during sleep. But instead of being depressed in sleep the hunger contractions continue with the same vigor as during the waking state, and in many instances with increased vigor. The increase in the gastric hunger contrac-

tions during sleep may be due to the elimination of all inhibitory impulses by way of the splanchnic nerves. But the absence of depression certainly indicates that the vago-gastric tonus mechanism, at least in man and the dog, occupies a unique position in the organism, a degree of independence of afferent impulses (exteroceptors) and central processes not known in the case of any other neuromuscular apparatus.

3. *The Effect of Cerebral States* (Emotional States, Intellectual Processes).—In the dog the cerebral processes of joy, fear, anger, eagerness (for food), attention, etc., cause temporary inhibition of the gastric hunger contractions. This inhibition takes place by way of the splanchnic nerves, not by a depression of the vagus tonus. This, again, points to an unusual independence of the vago-gastric tonus apparatus. The sight or smell of food on the part of the starving dog does not initiate or augment the gastric hunger contractions. Luckhardt has recently shown that when the sleeping dog dreams the gastric hunger contractions are inhibited in the same way that cerebral and emotional processes tend to inhibition of the contraction when the animal is awake.

In man, intellectual processes (attention, reading, figuring, arguing) have no distinct influence on the course of the hunger period. Actual anxiety causes temporary inhibition, probably through the splanchnics. We have not been in a position to make observations on the effects of actual anger, fear and joy, but there is no reason to believe that these processes act differently in man from that in the dog. In man we have paid particular attention to the effects of seeing and smelling palatable food, as it seemed *a priori* reasonable that the impulses generated by these stimuli might make more intimate connection with the vago-gastric tonus apparatus. Cannon assumes a " psychic gastric tonus " analogous to the " psychic secretion " of gastric juice. Glückmann states that the borborygmi are increased in rate and intensity on seeing and smelling palatable food. He ascribes this to increased gastric contraction. Extensive experiments on Mr. V. and on the author seem to show that is not the case. These stimuli neither initiate nor augment the gastric tonus and hunger contractions; so far as they influence them at all, it is in the direction of inhibi-

tion. One of the tests on the author might be given. Before beginning the five days' starvation period, our colleague, Dr. Luckhardt, was asked to bring in, unknown to the author, a tray of choice food in the midst of a hunger period. The arrangements being made, the matter was dismissed from the author's thoughts.

One o'clock of the morning of the fourth starvation day the subject was asleep and the record showed at this time a period of vigorous and regular hunger contractions. He was awakened to behold Dr. Luckhardt and an assistant enjoying a feast of porterhouse steak with onions, fried potatoes, and a tomato salad. The tray of edibles was placed not more than four inches from the subject's face and the delicious odor of the food filled his nostrils. He felt the hunger pangs as unusually intense, and there was considerable salivation. However, the gastric hunger contractions were not increased either in rate or intensity. In a few minutes, on the contrary, the hunger contractions became weaker and the intervals between them greater, and the period terminated by this gradual depression much sooner than it probably would have done in the absence of the dinner scene. This was undoubtedly due to local acid inhibition from copious secretion of appetite gastric juice.

When the hungry individual sees or smells good food the gastric hunger pangs are felt more intensely, although there is no change, or even when there is some decrease in the strength of the gastric hunger contractions. This is, therefore, a phenomenon of central reinforcement.

Our data on normal men and dogs seem incapable of any other interpretation than that the vago-gastric tonus apparatus, so far as it concerns the empty stomach, occupies a unique and physiologically isolated position, in the way of nervous control, while the inhibitory apparatus by way of the splanchnic nerves is readily influenced by central and reflex processes. We feel, however, that these observations must be extended to other groups of vertebrates as well as to such pathological cases in man in which there are indications of abnormalities of the vago-gastric tonus, before final explanations are attempted or speculation indulged in as to the usefulness of this physiological isolation.

This evidence for the physiological isolation of the hunger mechanism in the way of positive cerebral or central control is of interest in connection with the view that the cravings of hunger and appetite are subjective and largely a matter of habit, and that the periodicity or intensity of these cravings may be altered almost at the will of the individual. Chittenden states this view as follows: " The so-called cravings of appetite are largely artificial and mainly the result of habit. Any one with a little persistence can change his or her habits of life, change the whole order of cravings, thereby indicating that the latter are essentially artificial and have no necessary connection with the welfare or needs of the body. The man who for some reason deems it advisable to adopt two meals a day in place of three or four, at first experiences a certain amount of discomfort, but eventually the new habit becomes a part of the daily routine, and the man's life moves forward as before, with perfect comfort and without a suggestion of craving or a pang of hunger."

Our studies of the hunger mechanism seem to show that the above view is essentially wrong. In the normal individual the gastric hunger periods begin as soon as the stomach is empty and continue (in the absence of inhibitory processes) as long as the stomach is empty, irrespective of the time of day or night, and without reference to the time the individual is accustomed to eat. In individuals accustomed to eat the usual three meals in the day time and to sleep during the night, the gastric hunger periods are more frequent and usually more vigorous during the night (that is, during sleep) than during the day, provided, of course, the stomach is empty. In the normal individual the empty stomach exhibits periodic hunger activity, and there is no evidence to show that this primary automatism of the empty stomach is in the least influenced by eating one or by eating five meals a day. The basis for the view that the time of appearance of the " cravings of hunger " can be changed at will is probably to be sought in the fact that the milder hunger contractions do not enter consciousness as pangs of hunger if the individual's attention is directed into other channels. They are felt as hunger pangs if the individual's attention is directed towards food and eating. The

attention is thus directed, consciously or sub-consciously, about the time the individual is accustomed to eat. The periodicity of this subjective attention to the milder hunger cravings can probably be altered by training. But this applies only to relatively mild pangs of hunger. The more severe " cravings of hunger " caused by the gastric hunger tetanus rise above the threshold of consciousness, except in deep sleep or under conditions of a cerebral process involving intense interest. When an individual who is accustomed to eat three times a day turns to a régime of one meal a day, the quantity of food ingested in that one meal is much greater than that at any one of the three meals usually taken. The emptying of the stomach and the appearance of the pangs of hunger are correspondingly delayed. The view that prompt appearance and the persistence of the gastric hunger activity in the empty stomach have no relation to the actual need of the individual for food cannot be seriously maintained for the normal animal.

4. *The Influence of the Lower Brain Centres (Mid-brain, Medulla) on the Gastric Hunger Contractions.*—The most direct and at the same time the least objectionable method of attack on this problem is the section of the extrinsic nerves to the stomach, although this operation abolishes not only all direct influences from the brain of a motor or inhibitory type, but also the central reflexes (motor or inhibitory) that may be called into action through the sensory nerves in the stomach. The splanchnic nerves were sectioned through a median incision. The vagi nerves were sectioned 2–3 cm. above the diaphragm, thus leaving the fibres to the œsophagus, the heart and the lungs intact.

(*a*) *Complete Section of the Splanchnic Nerves.*—Observations were made on five dogs with complete sections of the splanchnic nerve on both sides. The longest period of observation after the splanchnic section was two months. Observations were in some cases begun two hours after the operation. When the records of these five dogs are viewed as a whole, it is clear that the *complete section of the splanchnic nerves in dogs increases the gastric tonus and augments the gastric hunger contractions.* The hunger contractions become more rapid and continuous, that is, there is less

evidence of the periodic groups with intervening periods of relative quiescence. It is not uncommon to observe contractions at the rate of about two per minute during an entire observation period of two to four hours. The section of the splanchnic nerves does not abolish the periodicity completely, however. It seems to be a question of relative degree of gastric tonus. If for any reason the tonus of the empty stomach is relatively low on any day, the hunger contractions are less frequent, and there is greater evidence of periods of relative quiescence. We desire to emphasize the fact that the above conclusion is based on the observations as a whole. Even the dogs with the splanchnic nerves sectioned showed on some days no greater tonus of the empty stomach or greater rate and persistence of the gastric hunger contractions than does the dog with these nerves intact. And occasionally a dog with the splanchnic nerves intact exhibits as great a degree of gastric tonus and rate and persistence of the gastric hunger contractions as the maximum observed in dogs with the splanchnic nerves out. This is to be expected, as by section of these nerves one eliminates only one (and in the normal probably one of the least important) of the factors in the motor activity of the empty stomach. The conditions that affect the stomach through the blood and through the vagi are still subject to the same variations as in the animal with the splanchnic nerves intact.

After complete section of the splanchnic nerves the psychic or reflex inhibition of the gastric hunger contractions is greatly diminished. The stimuli that cause anger, fear, pain, joy, or pleasure no longer lead to complete cessation of the hunger contractions. The maximum effect is a slight and transitory weakening of the contractions. It is, therefore, evident that the inhibitory fibres in the splanchnic nerves (and possibly also the secretory fibres of the adrenals) constitute the main efferent path in this type of inhibition. The slight degree of inhibition usually in evidence after section of the splanchnic nerves must be due to some central inhibition of the vagus tonus or to action of the few inhibitory fibres in the vagi.

Particular attention was given to the effect of seeing and

smelling food on the hunger contractions in these dogs with section of the splanchnic nerves, in order to determine whether these stimuli augment the tonus of the vagi and thus increase the hunger contractions. The results were negative. Even with the greater part of the extrinsic inhibitory fibres to the stomach eliminated, the sight, smell, and taste of food not only fails to inhibit or augment the gastric hunger contractions, but so far as these stimuli affect the stomach at all it is in the direction of inhibition of the hunger movements. The apparent increase in the intensity of the hunger pangs in man on seeing or smelling palatable food must therefore be essentially a central phenomenon of facilitation or "Bahnung."

(*b*) *The Section of the Vagi.*—Section of both vagi in the chest was made in three dogs, and after this operation observations on the gastric hunger contractions were continued for from two weeks to three months. Observations were made in some cases two hours after the vagi section. Section of the vagi leaves the empty stomach on the whole permanently hypotonic, that is, at least for a period of up to three months after the operation. The tonus of the empty stomach in these dogs varies somewhat from day to day, and occasionally the tonus may approach that of a dog with the vagi intact, but on the whole the tonus is permanently much lower than normal. This is evident not only from the observations by means of the balloon in the gastric cavity, but also on direct inspection and by palpation (introducing the finger through the fistula).

The hunger contractions of the empty stomach in these dogs are changed mainly in rate and regularity. The duration of each individual contraction is about normal, or on the whole less than normal. The long-drawn-out contractions or tetanus are rarely seen. But the intervals between the contractions vary on the whole from two to five minutes or even up to eight minutes. The strength or rather the amplitude of the individual contractions may appear greater than normal, evidently because the contractions start rather suddenly and without any marked preliminary increase in tonus, and the maximal contractions are so complete that all the air is forced out of the balloon. These

contractions may continue of fairly uniform amplitude and rate for two to three hours, that is, during a whole observation period. The contractions vary in strength and rate from day to day, and on some days they may be completely absent during the entire observation period (two to four hours).

The periodicity of the hunger rhythm is, on the whole, obscured, except on the days when the gastric tonus approached that in normal dogs. On such days the contractions appear at shorter intervals, and tend to fall into groups similar to those in normal dogs. Periods of gastric hunger contractions of normal rate and intensity have been observed as early as twelve hours after complete section of the vagi in the chest. The period of most powerful hunger contractions so far observed in any dog was recorded in one dog twenty-four hours after the vagi were sectioned. The dog had, during the four weeks preceding the vagi section, showed almost invariably the Type II rhythm. It was therefore a dog with unusually intense gastric motor activity. The complete section of the vagi causes on the whole less depression in dogs that exhibit great hunger contractions while the vagi are intact. The variations in the rate and intensity of the gastric hunger contractions in different dogs are therefore primarily due to individual variations in the condition of the stomach rather than to variations in the central innervation or the central inhibition.

In the dogs with vagi sectioned, but the splanchnic nerves intact, the " psychic" or reflex inhibition of the gastric hunger contractions is still in evidence, but the inhibition appears not to be so marked as when the vagi are intact. Accurate comparisons are, however, difficult to make because of the lowered tonus, and the usual long intervals between the hunger contractions after section of the vagi. We expected an augmentation of the inhibition through the splanchnics after the vagi section. Instead of finding this to be the case there actually appeared a gradual diminution in the influence of the splanchnic nerves on the empty stomach in the dog observed for three months after section of the vagi. It was not due to the regeneration of the vagi fibres, and consequent restoration of the vagus tonus. If further work should

establish this as a fact, we would have a significant instance of physiological readjustment,—either an actual diminution in the inhibitory impulses through the splanchnics in consequence of a dynamic readjustment in the central nervous system, or else an increased resistance, or "tolerance," to the splanchnic impulses and to epinephrine on the part of gastric motor mechanism.

(*c*) *Section of Both Splanchnic and Both Vagi Nerves.*—Complete section of the splanchnic and vagi nerves was made on four dogs, and observations made on the gastric hunger contractions for thirty to sixty days after the operation. The sections of the splanchnic nerves were made seven days after the section of the vagi. After this complete isolation of the dogs' stomachs from the central nervous system, there is practically a permanent hypotonus of the stomach except under conditions of prolonged starvation. The gastric hunger contractions are much the same as when the vagi alone are severed. The contractions are usually of great amplitude, but the intervals between the contractions are frequently longer than in normal dogs. The grouping of the contractions in periods is usually in evidence. These contractions of the isolated and empty stomach are present ten to twenty hours after the vagi section, and there is some improvement in the rhythm or an approach towards the normal tonus and contraction rate during the thirty to sixty days of observation. On the whole the hunger contractions of the isolated stomach conform to Type I. The Type II is rare, except during prolonged starvation. Short periods (two to three minutes) of incomplete tetanus are frequently seen, especially during prolonged starvation, and during the first half of the hunger period. It is, therefore, clear that all the essential characteristics of the hunger contractions of the empty stomach are determined by the local gastric mechanisms rather than by the character of the central innervation or the central inhibition.

Cannon has reported observations on the effects of vagi and splanchnic section on the gastric movements of digestion in cats. Section of the splanchnic nerves did not materially affect the movements of digestion; section of the vagi caused slowing and weakening of the peristalsis of digestion, but the normal rate of

peristalsis was practically restored in a few days. Combined vagi and splanchnic section left the digestive movements of the stomach practically normal, even shortly after the operation. It seems that section of the vagi or complete section of the vagi and the splanchnic nerves in dogs causes on the whole a greater change in the movements of the empty stomach than does the same lesion in cats in case of the movements of the filled stomach. This probably means that the tonus of the vagi plays a greater rôle in the movements of the empty than in the movements of the filled stomach. For it is not likely that there is such marked difference in the relative importance of the vagi in cats and dogs.

The changes in the character of the gastric hunger contractions after isolation of the stomach from the central nervous system seem primarily due to the persistent hypotonus. This is indicated by the fact that on days when the stomach of a normal dog shows relatively slight tonus, the hunger contractions approach the type shown by the isolated stomach, and on days when the isolated stomach exhibits tonus approaching that in normal dogs the hunger contractions tend to assume the normal type. Occasionally records are obtained from the empty and isolated stomach that practically demonstrate the above point. During a period of relatively slow hunger rhythm the tonus for some unknown reason may increase markedly for periods of varying length and during these periods the hunger contractions are identical in rate and character with those of the intact stomach in normal (strong) tonus. In one of the dogs with the vagi and splanchnic nerves sectioned, six days' fasting led to the appearance of periods of very great gastric tonus and during these periods (virtually periods of incomplete tetanus) the gastric contractions assumed the form of Type III.

However, the details of the changes in the hunger rhythm after isolation of the stomach from the central nervous system seem of minor importance in this connection. The essential point is that since the empty stomach, completely isolated from the central nervous system, does exhibit the typical hunger contractions, the efferent function of the gastric nerves is that of modifying or regulating a primarily automatic mechanism in the

stomach wall. In other words, the extrinsic nerves to the stomach play a rôle similar to that of the nerves to the heart in the regulation of the heart rhythm. Further analysis of the hunger mechanism must be directed primarily to the intrinsic neuromuscular apparatus of the stomach, and secondarily to the factors that control the vagus tonus.

II. REFLEX CONTROL OF THE HUNGER MECHANISM

The Inhibition from the Mouth in Man and Other Animals

Our gastric fistula man, Mr. V., offers an exceptional opportunity for studying the relations of certain conscious states, particularly those associated with foods and with eating, on the activities of the empty stomach. The œsophagus is completely closed at the level of the upper end of the sternum, so that nothing can enter the stomach from the mouth. The swallowing mechanisms are normal, and the man can swallow and hold in the œsophageal pouch about 25 c.c. of material. The gustatory (and olfactory) sense is normal. The senses of thirst and hunger are normal. He masticates his food in the usual way, and the chewing processes are accompanied by the normal conscious states. The masticated food is placed in a syringe and introduced into the stomach through the fistula, which does not involve any pain or discomfort, and the man is adjusted to this condition, as this has been his method of feeding for the last eighteen years. Because of the ample size of the gastric fistula the man may sit down at the dinner table, see, smell, taste and chew his food in the usual manner up to the point of introducing the food through the fistula, while tracings are being taken of a tonus and the movements of the stomach, and records made of the secretion of the gastric juice.

We know, particularly through the researches of Pavlov on dogs, and from many observations on man, that when appetite is present the sight, smell, taste (especially taste) of palatable foods cause a reflex secretion of gastric juice, the so-called "psychic secretion." The efferent nerve fibres for this reflex reach the stomach through the vagi. The more recent work of Cannon and others has demonstrated that the tonus of the stomach musculature is also primarily dependent on efferent nervous impulses

through the vagi. A certain degree of tonus is a pre-requisite for the digestion peristalsis or contractions in the empty stomach. The suggestion is therefore obvious that the same stimuli which lead to psychic secretion of gastric juice may at the same time cause an augmentation of the tonus and the contractions of the stomach musculature. Cannon postulated such a "psychic tonus," but no evidence for its extent has been recorded. It is a universal experience that the sight or smell (or even the memory) of palatable foods seems to induce hunger and appetite, or intensify these sensations if they are already present. The simplest explanation of this fact would be that the smell or taste of palatable foods initiates or augments the stomach contractions, thus increasing the hunger sensation by increasing the intensity of the gastric stimulation. The facts, at least in man and dog, are the very opposite of those demanded by this hypothesis.

There are two sources of error in experiments of this character. In the first place, the periods of contraction of the empty stomach vary in intensity and duration, and the intervening periods of relative quiescence vary in length. The periods of quiescence may be interrupted by occasional contractions. This being the case, the initiation of stomach contractions simultaneously with tasting palatable food during quiescence of the stomach, for example, may be a mere coincidence. An augmentation of the contractions seemingly due to tasting food during a contraction period may simply be the usual increase in strength of the stomach contraction during such period. In the same way, if tasting food towards the end of a contraction period should be followed by cessation of the stomach contractions, this apparent inhibition may be a coincident, the cessation of the contractions being "spontaneous" and not casually connected with the tasting of food. These difficulties were realized before the work was undertaken, as it was preceded by an extended survey of the "spontaneous" stomach movements when not interfered with experimentally. Because of the variability of the "spontaneous" stomach activity, the individual tests must be repeated a great number of times, and little or no significance can be ascribed to exceptional results.

A source of error more serious, because not so readily controlled, lies in certain subjective states of an inhibitory character. Pavlov found that while the sight and smell of palatable foods ordinarily caused "psychic" secretion of gastric juice in dogs when hungry, if the dogs knew from past experience that they were not to be permitted to eat the food, the same stimuli caused no secretion. We may have analogous conditions in regard to the stomach tonus and movements. It is possible that, no matter how great the hunger or appetite in man, the knowledge that the seeing, smelling or tasting food was part of an experiment might initiate cerebral processes of an inhibitory character. This source of error has been controlled in two ways: (1) In Mr. V. the mastication or tasting food was made part of his ordinary routine in preparing the food to be put into the stomach, and the man knew that as soon as the food was prepared it would be introduced into the stomach in the usual way. (2) Records were made of the presence or absence of the psychic secretion of gastric juice. If the tasting and chewing of food start a copious flow of gastric juice, we can infer that the tasting and chewing do not give rise to cerebral processes of an inhibitory character.

1. *The Inhibition of the Contractions of the Empty Stomach by Stimulation of the Gustatory End-Organs in the Mouth.*—The substances used for stimulation were sugar (solid and in solution), quinine in weak solution, sodium chloride (solid and in solution), weak solutions of acetic and hydrochloric acids. Tests were made at all stages of activity of the empty stomach. The results were uniform and practically identical for the four kinds of stimuli employed. If the substances were used in sufficient concentrations to affect the stomach activity, the effects were inhibitions of the tonus and contractions. These inhibitory effects follow promptly on placing the substances in the mouth, and disappear, on the whole, very soon after removing the substances from the mouth and rinsing the mouth with warm water. Quinine and the acid produced the longest inhibitory after-effects, probably because of the difficulty in completely removing these substances by rinsing the mouth with water.

This gustatory inhibition is, on the whole, proportional to

the strength of the stimuli (*i.e.*, the concentration of the substance), and varies inversely with the degree of the stomach activity. Thus a weak solution of acetic acid that produced distinct inhibition during the first stage of a period of hunger contraction when the individual contractions are relatively weak may have little or no effect when placed in the mouth during the tetanus stage of the contractions.

If the gustatory stimuli are weak and allowed to act in the mouth for five to fifteen minutes, the stomach "escapes" from the inhibition gradually. This is particularly true of sweet (sugar). Moderate strength of acids and quinine may hold the stomach in nearly complete inhibition up to fifteen minutes. The stimulating substances are, of course, gradually diluted by the secretion of saliva.

Are these gustatory inhibitions primary and relatively simple reflexes independent of the states of consciousness, or are they of the type of conditional reflexes, and therefore due to cerebral states of unpleasant effective tone? This question must be answered by experiments on lower animals with less development of the cerebrum and especially on decerebrated mammals, and on so-called "acephalic" infants.

2. *The Inhibition of the Tonus and the Contractions of the Empty Stomach by Chewing Indifferent Substances.*—We have been unable to obtain any definite evidence of inhibition of the stomach movements by the movements of mastication when the mouth is empty. But chewing what may be called indifferent substances, such as paraffin, gum, or straw, produces distinct inhibition. Most of the experiments were made by chewing paraffin. Most people can chew paraffin without any disagreeable or unpleasant sensation, or pleasant either, for that matter. Mr. V. said he "did not care for the paraffin," naturally. But he has no dislike for it. The chewing of indifferent substances produces, on the whole, less inhibition than do gustatory stimuli. The stomach "escapes" from the inhibition in a few minutes, even though the chewing is continued with uniform vigor. The chewing usually fails to produce any effects in the tetanus stage of the stomach activity. Inasmuch as the masticatory movements do not

cause inhibition if the mouth is empty, we may conclude that inhibition produced by chewing indifferent substances is initiated by mechanical stimulation of afferent nerve-endings in the mouth,

3. *Inhibition of the Tonus and the Contractions of the Empty Stomach by Chewing Palatable Foods When Hunger and Appetite are Present.*—Tests were made with all food substances palatable to Mr. V. and during all stages of gastric tonus and contractions, which imply all degrees of hunger and appetite. But most of the experiments were made with meats in the form of stews, fricassees, or pot roasts, fried eggs, and crackers or bread soaked in milk, soups or meat gravy. The results are uniform without exception. Chewing or tasting palatable foods inhibits the tonus and the movements of the empty stomach. The inhibition is in evidence within a few seconds after placing the food in the mouth, and may or may not continue for some time after removing the food from the mouth and rinsing the mouth with warm water. The inhibition is least in evidence during the hunger tetanus. In fact, we are uncertain whether the chewing of palatable foods is able to materially affect the stomach in hunger tetanus. It is difficult to determine whether cessation of the hunger tetanus that follows (usually not very promptly) on placing palatable food in the mouth is a "spontaneous" cessation, or due to inhibition from the mouth. The records show, however, that so far as the stimuli in the mouth affect the processes of the hunger tetanus, the influence is in the direction of inhibition.

The inhibition of the motor activity of the stomach by chewing palatable foods does not appear to have any after-effects in the nature of increased tonus or contractions. Some of the tracings do suggest a motor after-effect, but we are inclined to interpret them in a different way. These effects are obtained only when the tests are made during the relative quiescence of the stomach or at the beginning of a contraction period ("thirty-seconds rhythm"). Moreover, these results were not always secured even during these periods. It would, therefore, seem that these apparent augmentary after-effects represent the "spontaneous" initiation of a contraction period, or the gradual increase in the magnitude of the contractions characteristic of the periods of the thirty-seconds rhythm.

4. *The Factors Involved in the Inhibition of the Contractions of the Empty Stomach by Palatable Foods in the Mouth.*—Boldyreff has reported that the contractions of the empty stomach in the dog cease during the periods of "spontaneous" secretion of gastric juice. We know that tasting or chewing palatable foods leads to reflex or "psychic" secretion of gastric juice in mammals (including man). May not the inhibition described above be an indirect one due to the secretion of gastric juice, rather than a reflex inhibition of more direct character? This question has been investigated and settled. A rapid secretion of gastric juice is associated with cessation, partial or complete, of the stomach contractions in Mr. V. This is due, not to the processes of secretion, as such, but to acid stimulation of nerve-endings in the mucosa. When the chewing or tasting of palatable foods leads to copious secretion of gastric juice, this gastric juice is one factor in the accompanying inhibition of the stomach movements.

We know, from Pavlov's work on dogs, that the latent period of the "psychic" secretion is about five minutes. The latent period of the "psychic" secretion in man is shorter (2–3 min.). The inhibition of the stomach tonus and movements follows within a few seconds after placing the food in the mouth. Hence it is not an acid inhibition from the stomach. The same thing can be shown by some instances when the tasting or chewing of the food produces only a scanty secretion of gastric juice. The inhibition appears in the normal way, and the contractions reappear on removing the food from the mouth despite the slow secretion of gastric juice.

It seems that a certain quantity of gastric juice must accumulate in the stomach or the free hydrochloric acid in the stomach must reach a certain concentration before the acid inhibition takes place. Thus, if the period of chewing or tasting the palatable food is short (four to six minutes), the stomach contractions may reappear at the end of the stimulation in the mouth, and shortly afterwards again be inhibited by the acid gastric juice. This inhibition continues during the phase of rapid "psychic" secretion. When the psychic secretion is more copious, the reflex inhibition from the mouth merges into the acid inhibition from the stomach.

5. *The Inhibition of the Tonus and the Contractions of the Empty Stomach by Swallowing Movements.*—It has been shown by Cannon and Lieb for the dog that the movements of swallowing lead to a temporary inhibition of the tonus of the stomach. This inhibition is designated the "receptive relaxation" of the stomach. This inhibition is readily demonstrated in man. Mr. V. makes repeated swallowing movements with only enough saliva in the mouth to initiate the swallowing reflex, a prompt but transitory inhibition of gastric tonus and contractions is produced. The reader will recall that the swallowed saliva does not reach the stomach, but collects in the œsophagus pouch. Complete inhibition of the stomach contractions was never secured through the swallowing act, and when the stomach is in the condition of hunger tetanus, or in very strong and rapid contractions bordering on tetanus, the swallowing movements seem to have no effect on the stomach. The inhibition of the stomach tonus due to the act of swallowing is most readily demonstrated at the beginning of a period of hunger contractions.

6. *The Relation of the Reflex Inhibition of the Tonus and the Movements of the Empty Stomach from the Mouth to the Sensation of Hunger.*—The stimulation of the gustatory end-organs in the mouth, the chewing of indifferent substances, and the tasting and chewing of palatable foods abolish the sensations of hunger to the same degree that these measures inhibit the stomach contractions. The inhibition of the stomach activity and the cessation of the hunger pains run parallel. This conclusion is based on experiments on a number of men beside Mr. V.

In the dog, food or other substances in the mouth cause inhibition of the hunger contractions of the stomach. But since these manipulations disturb the animal, and induce salivation, and in many cases swallowing movements, the precise mechanism of the inhibition must remain in doubt until it can be investigated on dogs from which the cerebrum has been removed, since most of the dog's cerebral processes (pleasant or unpleasant) induce the same inhibition.

In the rabbit the sight, smell or taste of food, or the chewing (without swallowing) of such foods as cherries, carrots, apples,

carrot leaves moistened with sugar, acid or quinine do not inhibit the stomach contractions (Rogers). The same is also true for the guinea-pig (King). In the case of the single goat so far studied the chewing of ordinary food (hay, oats, carrots) appeared to increase rather than decrease the hunger contractions of the rumen.

In the pigeons Rogers encountered the same difficulties that we met in the dogs. Any disturbance of the normal pigeon inhibits the hunger contractions of the empty crop. And since it is not possible to put food or other substances in the mouth of these birds without more or less disturbance by the handling, we cannot be sure that the resulting inhibition proceeds from stimulation of nerves in the mouth. In the decerebrated bird visual and auditory stimuli do not inhibit the crop, but handling the bird, as in feeding or placing anything in the mouth, causes inhibition. If the disturbing factors other than the mouth stimulation could be eliminated it is likely that the mouth stimulation alone would cause little or no inhibition unless accompanied by swallowing. In the frog stimulation of the nerve-endings in the mouth by food substances, acids, or alkalies causes little or no inhibition of the empty stomach. This is true whether the frog is normal or decerebrated (Patterson).

It is thus evident that the marked reflex inhibition of the gastric hunger contractions from mechanical and chemical stimuli acting in the mouth of man is much less in evidence, although not entirely absent, in the lower mammals, birds and frogs. This leads us to suspect that in man and the higher animals where the reflex is preponderant it involves conscious cerebral processes. The question could possibly be settled by experiments on infants and on persons in deep sleep.

III. THE INFLUENCE OF STIMULATION OF THE GASTRIC MUCOSA ON THE HUNGER CONTRACTIONS

The character of the periodic and continuous motor activity of the empty stomach in man and other animals has been described. It has also been shown that the contractions of the empty stomach

give rise to the sensation of hunger or the "hunger pangs" by stimulation of afferent nerve-endings in the gastric mucosa. We have also seen that in man the hunger contractions of the stomach are inhibited, reflexly, by all stimuli acting on end-organs of taste and general sensations in the mouth cavity, so that in case of chewing palatable foods when in hunger we have the so-called psychic secretion of gastric juice preceded and paralleled by a psychic inhibition of gastric motility and tonus. It has also been shown that the hunger contractions persist in their essential character after section of the nerves connecting the stomach with the central nervous system. If we are to attempt to determine more specifically the cause of the hunger contractions our attention must be directed to the stomach itself. The contractions of the empty stomach may be due to several conditions.

1. *The Condition or the Stimulation of the Gastric Mucosa.*—The absence of food means absence of mechanical stimuli and cessation or diminution of the secretion of gastric juice, and hence a diminished acidity. Carbon dioxide may be secreted into the empty stomach and may act as the primary stimulus. Carbon dioxide and other gases may enter the stomach from the intestines, and act as stimuli. Succus entericus, pancreatic juice, and bile may enter the stomach and act as the primary stimulus through alkalinity or by means of specific substances such as the bile acids. The reader will recall that a number of workers maintain that bile facilitates the intestinal movements.

2. *The Condition of the Blood, Such as the Relative Concentration of Nutrient Substances, Tissue Metabolites, and Hormones.*—It is possible that the neuromuscular apparatus of the stomach is specially sensitized to slight variations in these substances. While we recognize the condition of the blood as a possible factor, it does not seem a probable one; in the first place, because the composition of the blood is on the whole more constant than the composition of the tissues, and because in young and vigorous individuals the hunger contractions of the stomach begin as soon as the stomach is empty, and while digestion and absorption is still in progress in the intestines, so there can be no lack of nutrient substances in the blood. In view of the relative con-

stancy of the composition of the blood serum, as shown by all past work, the existence of a periodic fluctuation in the concentration of any one substance in the blood parallel of the periodicity of the hunger contractions seems improbable.

3. *Nervous Impulses Through the Vagi.*—It is well known that the tonus of the stomach depends, in part, on impulses from the vagi, and that the stimulation of the peripheral end of the vagi induces strong contractions on the stomach whether empty or filled with food. It is also known that the stomach is capable of carrying out the movements of digestion to a fair degree of efficiency after section of both the vagi and the splanchnic nerves. In other words, the neuromuscular apparatus of the stomach seems to be primarily automatic, as regards the genesis of the movements of the digestion.

The experiment of sectioning the vagi does not prove this point, however. The experiment does prove the *plasticity* of the gastric motor mechanism. One would expect that the extrinsic gastric nerves bear the same relation to the movements of the filled and of the empty stomach. This phase of the problem cannot be studied in man. If it should develop that the periodic hunger contractions of the empty stomach are caused by periodic discharges through the vagi, the ultimate question of the cause of hunger would again become a problem of physiology of the central nervous system.

4. *A Primary Automatic Action of the Local Neuromuscular Mechanism of the Stomach.*—This can be established only by exclusion of the three other possibilities outlined above. A primarily automatic mechanism might still be influenced by the blood, by the extrinsic nerves and by local reflexes from the gastric mucosa. The periodicity of the automatic activity might be due, not to a parallel periodicity in any essential stimulus, but to some peculiarity in the metabolism of the stomach developed as a special adaptation, similar to periodicity in other organs. The absence of the hunger contractions during digestion, or possibly the modification of the hunger contractions into the movements of digestion, must, in this case, be due to specific inhibitory or regulatory impulses from the gastric mucosa.

Mr. V. is admirably adapted for determining the relation of stimulation of the gastric mucosa to the hunger movements, as the fistula is large enough to permit the balloon and connecting tube, and a tube for the introduction of liquids and gases, to be placed in the stomach simultaneously. The liquids and gases can be introduced with or without the man's knowledge. Furthermore, the contents of the stomach (fluid and gas) can be withdrawn for analysis at any stage of the hunger movements and without any material disturbance. But the results first obtained on Mr. V. have been abundantly confirmed on other persons. This can be done by simply introducing a small tube into the stomach in addition to the balloon with tube connection, so that substances can be put into the stomach without touching the mouth or œsophagus.

A. RESULTS OF EXPERIMENTS ON MAN

1. *The Action of Water.*—Water, at body temperature or nearly ice cold, inhibits the tonus and the hunger contractions of the stomach. The inhibition following the introduction of a glass of water (100–200 c.c.) directly into the stomach lasts on the whole only three to five minutes, and is never followed by any augmentation of the tonus or the hunger contractions. The cold water causes greater inhibition than the water at body temperature. If the water is introduced into the stomach during very intense hunger contractions ("hunger tetanus") there may be no perceptible inhibition. In other words, the degree of inhibition by water in the stomach is inversely proportional to the intensity of the hunger contractions present at the time the water is introduced. Water, warm or cold, introduced directly into the stomach during the period of relative relaxation and quiescence does not increase tonus or initiate a contraction period.

The statement that cold water causes on the whole greater inhibition than water at body temperature requires the following qualification: The record of the stomach movements were taken by means of an air-inflated balloon in the stomach cavity. Now, when cold water is introduced the water surrounds the balloon, at least partly, and cools the air in the balloon. This itself will

lower the tension somewhat, until the temperature is restored to that of the body by the warming of the water or by the passing of the water into the intestine. We do not think that this is a serious source of error, for this reason. A few experiments were made with water at 50° C. This causes greater inhibition than the water at 38° C. Water at 50° C. will, of course, increase the air tension in the balloon, yet the inhibition of the stomach tonus and movements is sufficiently marked to mask the effect of slight warming of the air.

How does water in the stomach produce this temporary inhibition? It goes without saying that in these experiments the water was not introduced fast enough to cause contractions by distention of the stomach walls, although this occurred unavoidably in a few instances. The only possible ways that water at body temperature can stimulate the nerve endings in the mucosa seem to be either by mechanical pressure or by osmosis. Cessation of the inhibition probably marks the passing of the water out of the stomach into the intestine, or the addition of sufficient salts to prevent stimulation by hypotonicity. The greater inhibitory action by cold water and by water above the body temperature is evidently due to stimulation of the protopathic temperature nerve-endings in addition to those acted on by pressure and osmosis.

It is clear that the action of water on the stomach mucosa is in the direction of inhibition of the hunger contraction. How can this be reconciled with the view that a glass of cold water induces or augments hunger? It is to be remembered that in these experiments the water had no chance to act on the nerve-endings in the mouth and the œsophagus. The alleged action of a cold drink on hunger and appetite is probably a reflex effect (cold) from the mouth and œsophagus. In the writer's own case a glass of ice water causes increased muscular tonus, sometimes even to the point of shivering and formication. This increased kinetic sensation probably acts in the way of "Bahnung" for the hunger sensation, if it is not actually a part of the hunger complex. Cannon and Washburne suggest that the effect of a cold drink on the hunger sensation is due to "the power of cold to induce contraction in smooth muscle." Although their meaning is not clear

to us, they probably have in mind the contraction of the stomach musculature. This could not come about by the cold acting on the stomach musculature directly. The reflex effects of cold water from the mouth and œsophagus are very complicated as regards the stomach, while cold water acting on the gastric mucosa directly causes inhibition, and cooling the frog's stomach causes depression and atony in proportion to the degree of cooling.

2. *The Action of Acids.*—All acids, or liquids containing acids, including normal human gastric juice, cause inhibition of the movements and the tonus of the empty stomach when introduced directly into the stomach cavity. No acid has been tested in stronger concentration than 2.0 per cent. The duration of the inhibition is on the whole directly proportional to the concentration and the total quantity of acid introduced. 200 c.c. of 0.5 per cent. of HCl will usually inhibit for a period of 25–30 minutes only.

This inhibition by acids can be made evident during all stages of activity of the empty stomach. If the acid is introduced during relative quiescence of the stomach the appearance of the next period of hunger contractions is delayed; if introduced during the active contractions these are abolished or depressed.

The duration of the acid inhibition is probably determined by three factors: (1) passing of the acid into the duodenum, (2) fixation and neutralization of the acid of the mucous gastric secretion, (3) neutralization by bile and intestinal juice which at times pass into the stomach through the dilated pylorus.

While it is a striking fact that gastric juice of full normal acidity (0.48–0.53 per cent.) and other acid solutions inhibit the hunger contractions, it does not follow that a neutral or alkaline reaction in the gastric cavity is a prerequisite for these contractions. During the strong contractions the stomach secretes a juice rich in mucin and combined HCl, but poor in free HCl. After the introduction of acids the contractions reappear before all the acid has passed out of the stomach or has been completely neutralized. And in case Mr. V. chews palatable food during a strong hunger period, the hunger contractions reappear before there is complete cessation of the psychic secretion of gastric juice. In

other words, the hunger contractions are not inhibited by weak concentrations of acids in the stomach. A neutral or alkaline reaction of the mucosa is not necessary for these contractions. If the food is sufficiently palatable and the mastication is continued long enough the inhibition produced reflexly from the mouth fuses with the acid inhibition from the stomach. If the food is not especially palatable or the mastication period brief, the contractions may resume on cessation of the chewing and then again be inhibited for a time during the period of most rapid secretion of the gastric juice. The degree of inhibition produced by normal gastric juice is the same as that caused by an equal quantity of hydrochloric acid of a concentration equal to the free acidity of the gastric juice. It would thus seem that the hydrochloric acid in the gastric juice constitutes the stimulus that leads to the inhibition.

This acid inhibition of the hunger contractions is of peculiar interest in connection with the neuromuscular mechanisms of these hunger movements and the gastric movements in normal digestion. In man the movements of the stomach in digestion are not inhibited by acids in the stomach, that is, at least not by acids in concentrations equal to that of the gastric juice. The fact that the intensity of movements of the antrum increases as the gastric digestion advances may even indicate that a certain degree of free acidity facilitates the movements of digestion. At first it occurred to us that since acid in the stomach inhibits the hunger contractions, but not the digestion contractions, the mechanisms involved in these two types of gastric activity are different, at least as regards the character of the afferent impulses from the gastric mucosa. But on further reflection it became apparent that this is not necessarily the case. For the digestive movements involve primarily the pyloric end, while the hunger movements (as studied by our method) involve the fundus of the stomach. It is possible that acid stimulation of the nerve-endings in the gastric mucosa leads, reflexly, to a temporary inhibition of the fundus and to peristalsis of the pyloric region of the stomach.

3. *The Action of Alkalies.*—The tests were made with sodium carbonate in concentrations varying from 0.2–1.0 per cent.; and

in varying quantities. In concentrations of 0.2 per cent. or less the sodium carbonate solution appears to have the same influence on the hunger contractions as equal quantities of water, that is a slight temporary inhibition. This inhibition is evidently due, not to the alkalinity, but to the bulk of the solution. In concentrations of 0.2 per cent. to 1.0 per cent. the degree of inhibition produced is on the whole directly proportional to the concentration and the quantity of the solution put into the stomach; 200 c.c. of one per cent. sodium carbonate causes about the same degree of inhibition as 200 c.c. one-half per cent. hydrochloric acid. It is thus clear that alkalinity has the same effect as acidity, only to a less degree; both acids and alkalies causing inhibition without any after-effect in the nature of augmentation.

The fact that 0.2 per cent. sodium carbonate has no more effect on the hunger movements than equal quantities of water seems to show that a slight alkalinity of the gastric mucosa is compatible with the hunger contractions of the empty stomach. It makes it also evident that the entrance of bile or intestinal juice into the stomach will have little or no effect on these movements, while any concentration that influences these movements produces inhibition.

4. *The Action of Local Anæsthetics.*—Solutions of some local anæsthetics were tested with the view of determining whether the sensory nerves in the gastric mucosa play only an inhibitory rôle in the processes of gastric hunger contractions. Phenol, chloreton, orthoform, quinine-urea-hydrochloride, and adrenalin chloride were used in quantities and concentrations compatible with absolute safety to Mr. V. It was not considered advisable to use cocaine. The solutions of the drugs were introduced in quantities of 100 to 200 c.c.

In the concentrations employed no specific action of any of the above substances could be determined. For example, 100 c.c. of phenol (dilution 1–10,000) has the same effect as 100 c.c. of water, that is, a slight temporary inhibition. The same applies to the other drugs. No appreciable anæsthesia of the gastric mucosa was produced by any of the drugs. It seems probable that the solutions of these drugs pass out of the stomach just as rapidly

as equal quantities of water, and hence do not remain long enough in the stomach to produce local anæsthesia. Because of the danger attending the use of local anæsthetics in strong concentrations in the stomach, this work was not carried further on man. It seemed, however, that adrenalin chloride introduced into the stomach even in considerable quantities could not be particularly injurious. But even in large quantities (100 c.c. of a dilution of 1–10,000) the adrenalin acting in the gastric cavity has no other effect on the hunger movements than equal quantities of water.

5. *The Action of Alcoholic Beverages.*—Tests were made with sour and sweet wines, beer, brandy, and pure alcohol. The taking of alcoholic beverages with the meals is a habit with many people. It is claimed by many people that a glass of wine, beer, or some mixture of alcohol taken before meals increases the appetite (and possibly the hunger). The writer is neither a total abstainer nor a habitual user of alcoholic beverages. But it is his experience that a glass of beer taken at meal times seems to awaken or increase appetite. This effect is rather immediate and therefore not due to the absorption of the alcohol. Pavlov has recorded an instance from his own experience where a drink of wine seemed to initiate the sense of hunger the very minute the wine reached the stomach. From inquiries as extensive as opportunities have permitted, we are inclined to believe that this apparent augmentation of hunger or appetite by alcoholic beverages is rather a common experience. In view of this fact we expected to find that these alcoholic beverages increased the tonus and the contractions of the empty stomach, since it is the tonus and the contractions of the empty stomach that gives rise to the hunger sensation. To our surprise the results proved to be the very opposite. Wine, beer, brandy, and pure alcohol introduced directly into the stomach inhibit the hunger contractions and the tonus of the empty stomach instead of increasing them. This is true whether these fluids are cold or at body temperature. If these alcoholic beverages are greatly diluted with water, a degree of dilution can be reached which has the same action on the empty stomach as equal quantities of water, although the specific beverage is readily detected when the

mixture is placed in the mouth. In no instance have we been able to make out any undoubted augmentation of the stomach tonus and hunger contractions after the inhibition period. In other words, alcoholic beverages when introduced directly into the empty stomach in quantities and concentrations that directly affect the tonus and the contractions of the stomach cause inhibition, and inhibition *only*.

The pure alcohol was never used in stronger concentrations than 50 per cent. The brandy was usually diluted with water, while the beer and wines were put in the stomach undiluted.

We have seen that acids in the stomach cause inhibition of the hunger contractions. Pure alcohol also causes inhibition. It is, therefore, evident that the alcohol and acids are primarily responsible for the inhibition following the introduction of alcoholic beverages into the empty stomach. For the sake of brevity we may designate it "the alcohol inhibition."

The duration of the alcohol inhibition varies directly with the quantity and concentration of the beverage introduced into the stomach. Thus 50–100 c.c. of 10 per cent. alcohol may inhibit the hunger contractions for one to two hours; or if introduced during a period of relative quiescence it delays correspondingly the onset of the next hunger period. 200 c.c. of beer causes inhibition for 3–60 minutes. The sour wines on the whole cause greater inhibition than the sweet wines, probably through their acids.

It must be stated that these alcoholic beverages were put into the stomach of Mr. V. and the other subjects, including the author, with their consent and without any protest, resentment, fear, or disgust on their part, which might account for the stomach inhibition. Mr. V. takes wine and beer occasionally. At times he bought his own choice of wine and beer and introduced into the stomach the desired quantities. The effect on the hunger contractions was always the same. We are therefore dealing with a characteristic alcohol and acid inhibition, and not with a masked "psychic" inhibition.

How are these results to be harmonized with the seeming stimulation of the appetite by alcoholic beverages taken by the

mouth? In the first place the local inhibitory action of alcoholic beverages in the gastric cavity is so marked and so invariable, that we feel confident that this is always the gastric effect of these beverages in man, whether taken normally by the mouth, or introduced into the empty stomach without coming in contact with the mouth or œsophagus.

Alcoholic beverages can, therefore, not initiate or increase hunger, since hunger is caused by the stomach contractions, and these are inhibited by the alcohol. Since most of the alcoholic beverages stimulate the end-organs of taste and smell as well as those of general sensibility in the mouth cavity and in the œsophagus, it is possible that this stimulation in some way augments or initiates *appetite* for food. If this is the case we have the singular condition of alcoholic beverages augmenting appetite and inhibiting hunger at the same time. There can be little doubt that cerebral states as modified by training and habit are also a factor in this apparent action of alcoholic beverages on appetite. It is certain that the individual's first taste of alcohol, beer, or sour wines does not focus his attention on food and eating.

If alcoholic beverages in the stomach caused as marked inhibition of the stomach movements in digestion as they do in the stomach movements in hunger even moderate drinking with meals would lead to acute indigestion. As this is not the case, it is evident that alcoholic beverages affect the mechanism of these two types of movements differently.

6. *The Action of CO_2 and Air.*—The action of CO_2 in the cavity of the empty stomach was studied in two ways: (1) By introduction of water charged with CO_2, and (2) by introduction of CO_2 gas. An excess of CO_2 in the blood of the abdominal vessels is supposed to augment the tonus of the digestive tract, although some recent work of Hooker throws doubt on this interpretation of the stimulating action on intestinal peristalsis by partial asphyxia. An excess of CO_2 is sometimes found in the gaseous contents of the empty or partly filled stomach. It is known, furthermore, that CO_2 in sufficient concentration acts as a powerful stimulus to the nerve-endings in such membranes as those of the mouth and nose, and of the cornea and conjunctiva.

CO_2 in the cavity of the empty stomach was at first considered a possible stimulus or cause of the gastric hunger contractions, but this hypothesis proved entirely erroneous. In so far as the CO_2 in the cavity of the stomach affects the hunger movements the influence is in the direction of inhibition.

Water saturated with CO_2 under pressure has practically no more effect than similar quantities of pure water. It produces the same degree of temporary inhibition without any after-effect of augmentation. As such carbonated water stimulates the nerve-endings in the mouth in the characteristic way, it follows that the nerve-endings in the stomach are less affected, by CO_2 than are the nerve-endings in the mouth.

When the CO_2 is forced into the stomach in the form of gas and under pressure, the results are complicated by the mechanical action of the gas in forcibly distending the walls of the stomach and raising the intragastric pressure, and hence increasing the pressure on the balloon in the fundus. A sudden and forcible distention of the stomach, no matter how produced, leads to a few strong contractions. This factor can be fairly well controlled by introducing the gas slowly. When this precaution is taken, the empty stomach can be considerably distended with CO_2 gas, without any marked action either on the tonus or on the hunger contractions. But the chemical effect of CO_2 so far as it is demonstrable at all, is in the direction of inhibition.

It will undoubtedly occur to the reader that this slight inhibition by the CO_2 may be an instance of "psychic" inhibition from the distress of an overdistended stomach. This possibility has been guarded against. In the first place the stomach was not distended to the point of painfulness by the CO_2. Furthermore, the stomach cavity was irrigated, so to speak, with the gas without raising the intragastric pressure perceptibly, by introducing the inlet tube to the cardiac end and allowing the gas to escape by way of the mouth. Under these conditions the same slight inhibitory effects were recorded without signs of primary or secondary augmentation. It is thus clear that in so far as CO_2 in the gastric cavity affects the gastric tonus and hunger contractions at all, the action is in the direction of inhibition. This is probably due to the acid stimulation of the nerve-endings in the mucosa.

The introduction of air into the empty stomach has no effect whatever on the tonus and the hunger contractions, provided the stomach is not overdistended by the air, or the air introduced rapidly and under such pressure as to cause sudden and forcible distention of the stomach walls. This leads to a few contractions. But the same thing is produced by sudden inflation of the balloon in the fundus. It is therefore purely mechanical. Oxygen in greater concentrations than that of the air has not been tried. But it is evident that the 20 per cent. oxygen of the air acts neither favorably nor unfavorably on the hunger movements.

The fact that nothing but inhibition is produced by substances acting on the gastric mucosa, suggests that this may be in every case a "psychic" inhibition masking any weak action that may be of a positive or augmentation type. The very consciousness that these substances were introduced into the stomach for experimental purposes might be the primary element in this possible psychic inhibition. That cerebral states may inhibit the gastric hunger movements is certain, from results both on man and on dogs. In one instance when preparing to introduce 200 c.c. of 0.5 per cent. acetic acid into the stomach in the midst of the period of powerful hunger contractions, Mr. V. somehow thought that we intended to introduce that much concentrated acid (or vinegar). As we were going about with the preparations it was noticed that the stomach contractions suddenly became very feeble. Mr. V. looked worried. We inquired if he did not feel right, and he asked if we intended to put all that vinegar into the stomach. "It will surely hurt me," he said. To assure him, the author drank half of the acid himself, and then asked him to take a mouthful of it. Then he laughed and said, "Oh, I thought it was pure vinegar." In two minutes after the mental stress and anxiety was over the hunger contractions returned to their normal rate and amplitude. The following facts speak against the possibility of the results being due to psychic inhibition:

(1) There was no evidence that Mr. V. or any of the subjects were in any way afraid, displeased, disgusted, or impatient with the experiments.

(2) The direct proportion between the quantity and the con-

centration of the substance introduced into the stomach and the degree of inhibition produced is contrary to the hypothesis of a psychic inhibition. The displeasure or disgust ought to have been practically the same on introduction of 0.1 per cent. and of 0.5 per cent. HCl, of 1 per cent. and 10 per cent. alcohol, as in most cases the subjects did not know the strength of the material used.

(3) In many cases the subject was purposely deceived as to the nature of the material, using water for acids and *vice versa*. The stomach reaction was invariably in accordance with the substance actually introduced.

We feel satisfied even on the basis of the tests on man that psychic inhibition plays no rôle in these results. But to meet the possibility once and for all, we have repeated and confirmed all of the above tests on dogs. The parallel on the two series on man and dog is complete. Well, may not psychic inhibition play a rôle in the tests on dogs? It does not, and for the following reasons:

First. The dogs could not have known either the difference between the substances introduced into the stomach or the different concentrations of the same substance.

Second. Tests were made during sleep and without the animal waking up. The results were the same.

Third. Psychic inhibition of the gastric hunger movements in dogs is invariably of much shorter duration than the inhibition caused by acids, alkalies, and alcoholic beverages acting in the stomach.

It is, therefore, clear that these results on man are fundamental facts in the physiology of the stomach and not primarily dependent on afferent impulses that enter consciousness.

7. *The Influence of the Inhibitions from the Gastric Mucosa on the Fundamental Rhythm of the Gastric Hunger Contractions*.—During the progress of this work it soon became apparent that these temporary inhibitions described above do not cut short a hunger period, but simply delay its culmination. The contractions that appear as the inhibition ceases are the continuation of the period temporarily checked by the inhibition. They are not the beginning of a new period. When the tetanus stage of the

hunger period is reached a stimulation of the gastric mucosa sufficiently strong to cause prompt cessation of the contractions seems to actually terminate the period, for when the contractions reappear they are not the incomplete tetanus or strong and rapid contractions of the culmination of the period, but the feeble and slow movements characteristic of the beginning of a period. By careful adjustment of the quantity and strength of the material introduced into the stomach during the first part of the hunger period and by renewing the inhibition on reappearance of the rhythm it is possible to lengthen a 30–40 minute period into a 90–120 minute period. In other words, the motor mechanisms of the hunger contractions may be compared to the spring of a watch. When the spring is wound up it will run the watch for a certain number of hours, and it makes no difference whether or not these hours are consecutive.

It seems to us that this fact has an important bearing on the question of the primary stimulus to the hunger movements. It seems to point to a primary automatism, peripheral or central, or both, relatively independent of the condition of the blood as well as of the afferent nervous impulses. The fact speaks particularly strongly against the hypothesis that the primary stimulus is to be sought in the condition of the blood. For example, if the primary stimulus is in some condition of the blood, this condition must be present and to a gradually increasing degree from 12.30 to 1 P.M. to parallel a hunger period beginning at 12.30 P.M. and ending at 1 P.M. And this condition of the blood must be absent from 1 P.M. to 1.45 P.M. when the stomach is relatively quiescent during that time. The hypothesis seems to be rendered untenable by the manipulations which do not, at least in some cases, involve any change in this hypothetical condition of the blood. The culmination of the hunger period may be delayed till 1.30 or 1.45 P.M., so that the strongest hunger contractions fall in the time when the blood does not stimulate the gastric mechanism in a way to cause hunger movements.

But what is the significance of this inhibition in the normal work of the stomach? The inhibition of the hunger contractions by mechanical and chemical stimulation of the gastric mucosa

prevents the appearance of these contractions during the period of gastric digestion. This negative control of the hunger movements from the stomach cavity is obviously a useful co-ordination. The primary or actual stimulus to the hunger contractions is, therefore, to be sought in the vagus tonus, in some condition of the blood, or in a primary automatism of the gastric neuromuscular mechanism. We have some evidence that the latter is the essential factor and that extrinsic nerves and the condition of the blood only modify the primary automatism. If this is the case the hunger contractions ought to appear as soon as the stomach is empty, or nearly empty, of food or other substances capable of stimulating the nerve-endings in the mucosa. We would also expect these contractions to be more or less continuous as long as the stomach is empty, at least in young and vigorous individuals, and when the condition of the individual as a whole does not lead to increased activity of the extrinsic inhibitory nerves (splanchnics). On this hypothesis the gradual tonus contraction of the gastric fundus *pari passu* with the progress of the gastric digestion represents the algebraic sum of the inherent automatism and the inhibitory effects from the gastric cavity. A gradual fatigue of the inhibitory mechanisms is probably also a factor, as we have abundant evidence, both in man and dog, of such "escape" of the stomach from inhibitory nervous processes.

We should probably look for the closest parallelism between the gastric hunger contractions and the absence of stimulation of the gastric mucosa in infants and young children, that is, before cerebral (and possibly gastric) habits relative to feeding have been established. We have made a close study of a healthy (bottle-fed) infant touching this point. It is well known that, other things being equal, the more food put into the stomach the longer time required for the completion of gastric digestion. If this infant (five months old) is given only four ounces of food he calls for more after about two hours. If he is given seven to eight ounces of the same food the call for more food is delayed for three to four hours. If he is given five ounces of the food at 6 P.M., he nearly always wakes up and calls for more at 12–1 o'clock; while if he is given as much food as he will take (7½ to

8½ ounces) at 6 P.M., he rarely wakes up and calls for food until 3–5 o'clock the following morning. There is evidently a close parallel between the time of the emptying of the stomach and the appearance of the hunger contractions. The more frequent calls for food during the day are obviously due to the fact that the gastric hunger contractions must reach a certain degree of intensity before they cause the soundly sleeping infant to wake up. This is certainly true in the case of dogs. A dog may sleep on peacefully and quietly during gastric hunger contractions of moderate intensity. When these contractions become very intense the dog moves or moans in his sleep and sometimes wakes up.

While we have made no observations on the action of acids, alkalies and alcoholic beverages on the gastric movements of digestion in man, it is well known that in the concentrations used these substances do not inhibit the digestion movements to the extent that they inhibit the hunger contractions. The movements of digestion are primarily concerned with the pyloric region, while the hunger contractions involve the cardiac and fundus region. Evidently these two regions of the stomach react differently to local chemical stimulation of the gastric mucosa.

In view of the fact that acids as well as normal gastric juice inhibit the gastric hunger contractions, one might expect that persons having gastric hypersecretion should experience little or no true hunger sensations or pangs of gastric origin. At the same time we must consider, in cases of prolonged hypersecretion, the possibility of a readjustment of such a character that the acid stimulation of the mucosa causes less inhibition than is the case in the normal stomach.

B. RESULTS ON DOGS

The work on man led to the conclusion that any substance capable of stimulating the nerve-endings in the gastric mucosa causes inhibition of the tonus and hunger contractions, and inhibition only, as there is no evidence of any increase in the gastric tonus or hunger contractions following the primary inhibition. The experiments on dogs were undertaken primarily to determine the character of this reflex, that is, whether central, or local, or

both. The liquids were introduced into the stomach through the fistula by means of a soft rubber tube so that swallowing acts, and the stimulation of nerve-endings in the mouth, the pharynx, and the œsophagus, were completely eliminated.

1. *The Action of Water, Acids, Alkalies, and Alcoholic Beverages.*—The observations were made on six dogs with all of the extrinsic gastric nerves intact, on six dogs with the splanchnic nerves cut; and on four dogs with complete section of both of the vagi and the splanchnic nerves. The results on the normal dogs are practically identical with those on man. Gastric juice (human and canine), weak acids and alkalies, brandy, wines, and beer introduced directly into the empty stomach during hunger contractions produce immediate inhibition of the gastric tonus and contractions. Thus, the same quantity of gastric juice or wine seems to cause more prolonged inhibition in dogs showing Type I than in the dogs showing Type III hunger rhythms. The duration of these inhibitions can best be studied in the dogs showing the Type II and III hunger contractions, as these two forms are practically continuous, so that the errors from spontaneous periods of relative quiescence are eliminated. In normal dogs showing Type II and III contractions, 25 c.c. gastric juice or 0.5 per cent. HCl usually causes complete inhibition for 20 to 30 minutes. The return of the hunger contractions is always gradual. 25 c.c. of beer will inhibit for 15 to 25 minutes. In one case 50 c.c. of beer caused complete inhibition for one hour.

If these substances are introduced into the stomach of dogs during a period of relative quiescence and tonus relaxation, the only effect appears to be a still greater tonus relaxation and prolongation of the quiescent period. In some cases one or two hunger contractions follow immediately on introducing the material into the stomach. We are inclined to attribute these contractions to the mechanical distention of the stomach wall rather than to stimulation of nerve-endings in the mucosa. This phenomenon was never observed when the stomach was in strong tonus and hunger contractions.

2. *The Action of CO_2.*—The influence on the hunger contractions of CO_2 in the stomach cavity is the same in dog and man.

The experiments on dogs were made with water saturated with CO_2 and with CO_2 gas. When the gas was employed, at times enough of it was passed into the stomach via the fistula to cause escape of the gas through the œsophagus. The water saturated with CO_2 has practically the same action as ordinary water, that is a slight temporary inhibition without any after-effect of the nature of increased tonus or contractions. This is true whether the carbonated water is introduced during active hunger contractions or during relative quiescence. The CO_2 gas usually initiates some contractions if introduced into the stomach during a period of quiescence. This is evidently due to mechanical distention of the stomach walls and not to chemical stimulation of nerve endings in the mucosa. If the empty stomach is in vigorous tonus and hunger contractions the CO_2 gas causes a slight temporary inhibition without any stimulating after-effect. This temporary inhibition is in all probability due to a weak acid stimulation in the endings in the mucosa.

3. *The Effects of Complete Section of the Splanchnic Nerves.*—The inhibition of the gastric tonus and hunger contractions by acids, alkalies, alcohol, etc., in the stomach cavity persists after section of the splanchnic nerves, but it is on the whole less complete and of shorter duration than in dogs with all the extrinsic gastric nerves intact. This applies to all substances used in this series of experiments. When as in the present series, the test with each substance is repeated at least ten times on each animal, some variation in the intensity and duration of the inhibition appears. That is to be expected, because the degree of inhibition depends on several variable factors, such as the excitability of the nerve-endings in the mucosa, the excitability of the Auerbach plexus and of the central nervous system, the tonus of the stomach, etc. It is therefore true that the most pronounced inhibition observed after section of the splanchnic nerves may be as marked as the feeblest inhibition obtained in the normal dogs. But when all the results in the two series of dogs are compared there is no question but that *section of both the splanchnic nerves diminishes the inhibition following chemical stimulation of the gastric mucosa by acids, alkalies, alcohol, etc.*

Several explanations of this fact suggest themselves: (1) Since section of the splanchnic nerves in dogs increases on the whole the tonus and the hunger contractions of the empty stomach, the diminished inhibition may be due to this greater vigor of the stomach, rather than cutting the efferent path of a long reflex. We do not think that this is the main factor, because the typical marked inhibition is obtained in normal dogs even when the stomach shows as vigorous tonus and hunger contractions as the maximum shown by dogs with the splanchnic nerves severed. Moreover, the inhibition is still incomplete in dogs with cut splanchnics, showing relatively feeble hunger contractions. (2) The substances stimulate afferent vagi nerve-endings in the mucosa, and the afferent vagi impulses via conscious or subconscious centres finally stimulate the efferent inhibitory neurones in the splanchnic system. It is well known that the vagi carry afferent fibres from the stomach mucosa and that the splanchnic nerves carry inhibitory fibres to the stomach. The present experiments give the first intimation that the afferent vagus and the efferent splanchnic systems are so intimately associated in gastric motor reflexes. It is possible that the reflex also involves the adrenal glands, so that the above inhibition is to be accounted for, in part, by the depressor action of an increased output of epinephrin.

4. *The Effect of Section of the Vagi Nerves and of the Vagi and the Splanchnic Nerves.*—When all the records are compared it appears that section of the vagi nerves alone or section of both the splanchnic and the vagi nerves diminishes the inhibitory reflex from the gastric cavity on the whole more than does the section of the splanchnic nerves alone. A fact of greater importance, however, is the persistence of the reflex after complete isolation of the stomach from the central nervous system. *The inhibition is therefore a primary, a local reflex.* The increased diminution of the inhibition after the vagi section may involve two mechanisms. It is well known that the vagi contains some efferent inhibitory fibres to the stomach mechanism, and may be, together with the splanchnic inhibitory fibres, involved in the long inhibitory reflex. But since the gastric tonus fibres in the vagi and the gastric inhibitory fibres in the splanchnic nerves are practically antagonistic,

it is highly probable that afferent influences leading reflexly to the stimulation of the inhibitory neurones lead at the same time to the inhibition of the tonus of the motor neurones.

You may object that we are now discussing inferences that do not necessarily follow from the facts so far at hand. The facts, in brief, are these. The inhibition of the tonus and the contraction of the empty stomach by stimulation of the gastric mucosa persist after isolating the stomach from the central nervous system, but the inhibition is diminished in intensity and duration after section of the splanchnic nerves, and somewhat more so after section of the vagi nerves. It has been shown that section of the vagi leaves the stomach on the whole permanently hypotonic, except during prolonged starvation, although there seems to be a gradual improvement in the efficiency of the local tonus mechanism. Is it not possible that the lessened inhibition after the vagi lesion is due to the depression of the inexcitability of the local afferent nerve-endings in the mucosa or depression of the local reflex centre similar to the tonus depression? Our experiments do not exclude this possibility, but the results on the dogs with only the splanchnic nerves severed show conclusively that it is not the sole factor. For in these dogs there is no gastric hypotonus, and yet the inhibition from the gastric mucosa is diminished.

Another possibility has occurred to us. When the same quantity (25–50 c.c.) of acids, alkalies, or alcoholic beverages is introduced into the stomach in tonus and into a stomach in hypotonus, it seems likely that the solution will come in contact with more of the mucous membrane in the tonic than in the atonic stomach. This might result in less inhibition in the case of atonic stomach from the mere fact of stimulation of less of the afferent nervous mechanism. We have tested this possibility by introducing a greater quantity of the respective solutions in the hypotonic stomach. But if 25 c.c. of acid or beer fails to produce complete inhibition, 50 c.c. of the same liquid usually also fails. This is to be noted, however, that the depression of inhibition following splanchnic and vagi section is most marked for a week or two after these nerve lesions are made, and there is a distinct

tendency in the efficiency of the local reflex *pari passu* with the improvement of the local tonus mechanism. This is probably an instance of readjustment of local reflex mechanisms to a fair degree of efficiency in the absence of central tonus and accessory central long reflexes.

The experiments on man and on normal dogs led to the conclusion that contractions of the empty stomach cannot be induced by the stimulation of the gastric mucosa, that such stimulation causes inhibition only. It was noted that one or two contractions occasionally may follow immediately on the introduction of these liquids into the stomach, but it seemed probable that these contractions were due to the mechanical distention of the stomach walls rather than to the chemical or mechanical stimulation of the nerve-endings in the mucosa. These initial contractions following the introduction of acids, alkalies, or alcoholic beverages into the stomach occur more frequently in the hypotonic stomach isolated from the central nervous system. This is true even when special care is taken to introduce the substance slowly so as not to cause sudden distention of the stomach walls. We are not yet satisfied that this primary motor response is actually due to stimulation of nerve-endings in the mucosa. If it is, there must be in the mucosa a few afferent nerve-endings of the excitatory type, but the afferent inhibitory nerve-endings are so much more numerous that the influence of the former group is completely submerged by the latter except occasionally when the stomach is hypotonic. Or else local afferent nerve-endings in the mucosa are all of one type, but the type of reflex produced by this stimulation may depend in part on the tonus condition of the reflex centres (Auerbach plexus).

The local and long reflex mechanisms governing the tonus and the hunger contractions of the empty stomach demanded by the above work on dogs are diagrammatically represented in Fig. 8. It may be noted that this diagram is not intended to represent all the afferent gastric nerve components, such as those acting in various ways on consciousness, on the vaso-motor centres, etc. The adrenal glands are indicated simply as a possible factor, because conclusive data have not yet been obtained on that point.

C. RESULTS ON OTHER SPECIES

Rogers found that water, weak alcohol (10 per cent.), weak acids (0.2–0.4 per cent. HCl), sugar solutions, fruit juices, etc., introduced directly into the stomach in rabbits cause temporary inhibition of the hunger contractions, but have no apparent effect on the digestion peristalsis when introdued in the filled stomach. In the guinea-pig King failed to obtain definite inhibition of the hunger contractions by chemical stimulation of the mucosa. In these experiments it is possible that the stomach was not completely empty, hence the resistance to local chemical stimulation, as Rogers and Hardt found that even in man the tonus rhythm of the fundus that is present during the digestion peristalsis is much more resistant to inhibition from chemical stimulation of the mucosa than is the very same tonus contraction in the empty stomach of the same individual.

Water, weak acids, etc., introduced directly into the empty crop of pigeons cause inhibition of the hunger contractions. In the bullfrog water, weak acids, weak alkalies, etc., inhibit temporarily both the hunger contractions and the digestion peristalsis. In the frog the inhibition from the gastric mucosa is much more marked than that produced by chemical stimulation of the nerve-endings in the mouth (Patterson).

The inhibitory reflexes from the gastric mucosa to the gastric musculature are thus present in all animals so far studied. The mechanism is probably present in all animals with a well developed stomach. But the efficiency of the reflexes varies in different species, and in the same species or individuals they vary with the condition of the stomach (filled or empty).

IV. INHIBITORY REFLEXES FROM THE INTESTINAL MUCOSA TO THE EMPTY STOMACH

We have seen that the tonus and contractions of the empty stomach (man and dog) are temporarily inhibited by stimulation of nerves in the mouth, in the œsophagus, and in the gastric mucosa itself. Can the tonus and hunger contractions of the empty stomach be influenced reflexly by stimulation of the intes-

tinal mucosa? The answer to this question might explain the diminution or abolition of hunger by the introdution of chyme into the intestine. If such reflex relations exist, it is obvious that the intestinal mucosa must be an important factor in the control of the gastric tonus and hunger mechanism.

Boldyreff reports that acids in the intestine inhibit the periodic activity of the empty stomach. The inhibition was not obtained by water or alkaline solutions. In fact Boldyreff appears to imply that the periodic contractions of the empty stomach may be initiated by the introduction of a solution of 0.3 per cent. Na_2CO_3 into the intestine. He therefore concludes that the reflex inhibition is due to an acid stimulation of nerves in the intestinal mucosa. If chemical stimulation of the intestinal mucosa induces increased intestinal tonus and contractions we would expect the increased motility of the intestines to cause some inhibition both of the digestion peristalsis and the hunger contractions of the stomach according to the interesting theory of gastro-intestinal co-ordination recently advanced by Alvarez.

In our work we used 24 young female dogs. Intestinal fistulæ were made by Abbe's lateral anastomosis in the first loop of the small intestine below the pancreas, the cephalad end being sutured into the abdominal wall and left open to the exterior. The gastric fistulæ were made after recovery from the first operation.

In another group of dogs a Tiery fistula was made, but no gastric fistula, the recording apparatus being introduced into the stomach through the œsophagus.

In the third group the gastric fistula was made near the pyloric end of the stomach. Through this fistula a small stomach-tube was passed through the pylorus into the small intestine for varying distances. This tube was kept in the gut throughout the experiment for the introduction of the liquids into the intestine. The recording balloon was passed into the stomach either through the gastric fistula or through the œsophagus.

In the last group of dogs the vagi and splanchnic nerves were cut, and after recovery from the operation, gastric fistulæ were established in the antrum pylori. In all tests on this group the fluids were introduced into the intestine by means of a tube passed

through the pylorus, and the stomach balloon was passed down through the œsophagus.

The following solutions were introduced into the intestine in 10 c.c. quantities, in most cases at body temperatures: Normal gastric juice (dog and man); 10 per cent. Witte's peptone in 0.2 per cent. HCl; pepsin in 0.2 per cent. HCl; hydrochloric acid (0.1 per cent. to 0.5 per cent.); saturated H_2CO_3 solution; neutral olive oil; fresh milk; water; mechanical stimulation of the intestinal mucosa (glass rod or rubber tube).

RESULTS

When the vagi and splanchnic nerves are intact all mechanical and chemical stimulations of the intestinal mucosa cause inhibition of the gastric tonus and hunger contractions. The effect of a purely mechanical stimulation (rubbing the mucosa with a glass rod or rubber tube) is the most transitory. In general pure gastric juice and the 0.5 per cent. HCl cause the longest inhibition. The acid-peptone solution follows these closely. The weaker acids produced inhibition of less duration. Saturated carbonic acid solution did not give quite so distinct an inhibition as the other acids. Inhibition with pure gastric juice and the acid-peptone mixture varied in duration from three to twenty minutes, depending apparently largely on the condition of the animal at the time. The sodium carbonate solution caused inhibition of less duration than acid mixtures, but of longer duration than the water or the neutral mixtures in general. However, the longest inhibition obtained in any one experiment was produced by 10 c.c. of milk in the gut. In this case the inhibition lasted thirty minutes. Ordinarily, neutral solutions produced a longer inhibition than the mechanical stimulation by moving the soft rubber tube in the intestinal fistula.

In the animals with the vagi and splanchnic nerves severed the above substances still caused reflex inhibition of the empty stomach from the intestinal mucosa, but the latent period of the inhibition was greatly prolonged, the degree of the inhibition less, and the duration of it much shorter than in the normal animals. It is therefore clear that this inhibition of the tonus and con-

traction of the empty stomach by chemical and mechanical stimulation of the intestinal mucosa involves both long or central and short or local reflex paths, a situation similar to that found in the gastric mucosa itself.

We may conclude, then, that gastric juice, chyme, acids, alkalies, water, milk and oil introduced into the small intestine inhibit gastric hunger contractions and gastric tonus for varying periods. This inhibition is due partly to mechanical, partly to chemical stimulation of the intestinal mucosa. The chemical stimulation produces the greatest effect. This inhibition takes place primarily by the "long" or central reflex path, but "short" or local reflex paths in Auerbach's plexus are also involved.

The precise rôle of these reflexes in the control of the gastric hunger mechanism in the normal animal must be determined by further investigation. They are probably factors in the diminution or absence of hunger in cases of enteritis, intestinal obstruction, constipation, appendicitis, and cholecystitis.

V. THE INHIBITION OF HUNGER BY SMOKING AND BY PRESSURE ON THE ABDOMEN (CONSTRICTION OF THE BELT)

It is generally held to be true that smoking shortly before a meal leads to depression of hunger and appetite. It is also a common belief that strong pressure on the abdomen ("tightening the belt") decreases or relieves the hunger sensation, at least temporarily. We are now in position to test the correctness of these beliefs by decisive experiments, as regards the influence of these measures on the objective hunger contractions and the subjective hunger sensations.

Depression or inhibition of hunger by smoking is rendered probable by the fact that, at least in man, anything which stimulates the sensory nerve-endings in the mouth and in the gastric mucosa inhibits the gastric hunger contractions in direct proportion to the intensity of the stimulation. Smoking stimulates the nerve-endings in the mouth in varying degrees according to the kind of tobacco used. Smoking frequently involves stimulation of the nerve-endings in the gastric mucosa owing to the swallowing of saliva containing nicotin, oils, tannic acid, and other irritating

substances. Smoking may also act on the hunger mechanism in a third way, that is, through absorption of nicotin and other products of the combustion. The third possibility has not been investigated. It is well established, however, that even small quantities of nicotin in the blood lead to nausea and vomiting. Nausea and vomiting are accompanied by atony of the gastric fundus, which insures absence of hunger contractions and hunger sensations.

The effects of smoking on the gastric hunger contractions were first studied on Mr. V., our young man with the permanent gastric fistula. In his case smoking (cigars) leads invariably to inhibition of the hunger contractions. But Mr. V. is not an habitual smoker. It is, therefore, possible that the results obtained on him were simply due to the condition of nausea or disgust that smoking usually produces in the novice, and hence not applicable to persons used to smoking. The tests were repeated on several habitual smokers. In so far as smoking influences the gastric hunger contractions this influence is in the direction of inhibition. This inhibition appears to depend on the intensity of stimulation of the nerve-endings in the mouth, a cigarette or "mild" cigar causing only slight inhibition, while a "strong" cigar or pipe causes complete and prolonged inhibition even when the gastric hunger contractions are at their maximum.

If the cigar or pipe causes very strong stimulation of the nerve-endings in the mouth, the inhibition of the hunger contractions may continue from five to fifteen minutes after the cessation of the stimulation. Thus, even a brief period of smoking may suppress an entire hunger period.

The subjective sensation of hunger is diminished or abolished parallel with the gastric hunger contractions. But it seems to the authors that even a "mild" smoke diminishes the sensation of hunger rather more than one might infer from the slight depression of the contractions. This is probably due to the deviation of attention, the smoking acting partly as a "counter-irritant."

Smoking inhibits the gastric hunger contractions. It is practically certain, even in the absence of direct experiments, that moderate smoking does not inhibit the gastric movements of diges-

tion. The reason for this difference in the action of the same condition on the empty and on the filled stomach is not clear.

The experiments with constriction of the belt were made on three normal men. The tests were made with the subject standing up, sitting, and lying on the back, and at all stages of the gastric hunger contractions. Strong contraction of the abdominal belt leads nearly always to inhibition of the gastric hunger contractions of weak or moderate strength, lasting from five to fifteen minutes. The inhibition may be partial or complete, but in either case the hunger contractions reappear despite the continued pressure of the belt. This inhibition is obtained even when the belt constriction is moderate so that no discomfort or pain is produced. When the gastric hunger contractions are strong (the middle of a hunger period), constriction of the belt never causes complete inhibition. But so far as the increased abdominal pressure affects the hunger contractions the influence is in the direction of inhibition. The individual hunger contractions are weakened without suffering much change in the rate. Frequently, however, even a belt constriction that caused considerable discomfort has practically no influence on the hunger contractions, particularly if the subject is lying down.

When the gastric hunger contractions are at their maximum in rate and amplitude, as is ordinarily the case near the end of a hunger period, no amount of belt constriction seems to influence the contractions. When this stage of the hunger period is reached the hunger pangs run their normal course in the presence of even painful belt pressure.

All three subjects agreed that the belt constriction appeared to diminish or interfere with the hunger sensation to a greater extent than seemed warranted from its effect on the hunger contractions. Several factors are probably involved in this discrepancy. (1) The belt constriction distracts the attenion from the hunger impulses by stimulation of nerve-endings in the viscera, especially those of the peritoneum. (2) Strong pressure on the abdomen from without appears to induce, temporarily, a condition simulating in a feeble way the complex sensation of satiety.

According to R. Lennhoff,[1] hunger and appetite are appeased

[1] Quoted in Jour, Amer. Med. Assoc., 1913, lx, p. 41.

with a less quantity of food when the belt is constricted than when the intra-abdominal pressure is regulated solely by the tonus of the abdominal muscles. Lennhoff ascribes this to depression of hunger and appetite by the pressure of the belt. Lennhoff's observation is probably correct, but his explanation is erroneous. In a normal person the actual hunger contractions and hunger sensations are stopped by the first few morsels of food swallowed, while this may actually increase the appetite through stimulation of nerve-endings in the mouth and in the mucous membrane of the œsophagus and stomach. This appetite sensation is gradually counteracted by the sensation complex of satiety, which depends in part on the distention of the stomach with corresponding readjustment of the tonus of the abdominal muscles. This feeling of fulness, which appears to be referred to the abdomen as a whole, is probably developed with less intake of food when the abdominal wall is mechanically prevented from relaxing owing to the pressure of the belt.

We have practically nothing but conjectures to offer in way of explanations of the mechanisms involved in the above inhibition of the gastric hunger contractions by strong pressure on the abdomen. Strong pressure on the abdomen causes temporary inhibition of the gastric hunger contractions in dogs, but the manipulation greatly disturbs the dogs, and disturbance from any cause leads to a temporary inhibition of the empty stomach in dogs with the splanchnic nerves intact. In dogs with the splanchnic nerves sectioned on both sides, strong pressure on the abdomen causes no distinct inhibition of the gastric hunger contractions. This points to the conclusion that belt constriction causes gastric inhibition, not by direct pressure on the stomach, but by direct stimulation of inhibitory nerves, or by mechanical (or sympathetic) stimulation of the adrenal glands,[2] but through long reflexes. Belt constriction involves stimulation of cutaneous nerve-endings, but the stimulation of the tactile nerve-endings in the skin alone does not lead to this inhibition. The afferent

[2] Stewart, Rugoff and Gibson have recently shown that there is an increased liberation of epinephrine from the adrenal glands on massage of the glands. Jour. Pharm. and Exp. Therap. 1916, viii, 205.

path of the reflex must therefore involve abdominal proprioceptors. The splanchnic nerves probably constitute the efferent path of the reflex. We do not wish to be understood as denying the existence of local inhibitory mechanism that may be stimulated by mechanical manipulation of the abdominal organs, but our results indicate that strong belt constriction is not a sufficient stimulus for such local mechanisms. The solution of this question is of practical importance in connection with the employment of mechanotherapeutic measures to control motor disorders of the alimentary tract.

VI. THE INFLUENCE OF PHYSICAL EXERCISE AND EXTERNAL COLD ON THE HUNGER MECHANISM

So far we have been unable to initiate or augment the gastric hunger contractions in man or experimental animals by any sensory stimulation or central nervous processes. We have seen that so far as these nervous processes affect hunger it is in the direction of inhibition. It is singular, indeed, that the inhibitory mechanisms are so readily called into play, while motor reflexes are either inaccessible or lacking, especially since the utility of some of the inhibitory reflexes are open to question. From the point of view of biological adaptation we might expect the vagogastric tonus to be directly affected by voluntary muscular activity and by exposure to cold, since both conditions involve increased oxidation and consequently increased need of food.

Muscular activity may augment the gastric hunger activity by increasing the vagus tonus as well as by chemical changes in the blood. The same applies to stimulation of the cold nerve-endings of the skin. However, it is probable that if these conditions cause increase in the vagus tonus reflexly this response is more prompt than that induced by the changes in the blood following the increase or decrease in body metabolism due to stimulation. It is generally recognized that exercise, cold climate, and cold baths increase appetite and hunger. It does not follow that these conditions actually augment the gastric hunger contractions. The increase in hunger and appetite may be only apparent, that is, a condition of increased excitability of parts of the central ner-

vous system, so that the afferent impulses that give rise to the sensation of hunger and appetite produce a greater central effect. If the gastric hunger contractions are actually increased, this may be due to changes in the blood rather than to increased vagus tonus.

It is well known that exposure of the skin to cold (as by bathing in ice water) may induce contracture of "cramps" of the digestive tract. This is especially the case during the height of gastric and intestinal digestion. These cramps and contractures may be the result of circulatory disturbances or of changes in the blood rather than a direct reflex effect. Central processes are also able to induce contraction of the large intestine and the rectum, as shown by involuntary defecation in cases of great anxiety or fear.

EXPERIMENTAL PROCEDURE

Experiments on Dogs.—Dogs with simple gastric fistulas were trained to run in a treadmill. When trained to run without urging or interference, records were taken of the contractions of the empty stomach so as to determine (1) whether muscular activity induces hunger contractions in the quiescent stomach, and (2) whether muscular activity augments the hunger contractions of an active stomach.

The hunger contractions of the stomachs of dogs were recorded for 2 to 4 hours after a day's fast, the dogs being taken direct from the kennel without being exercised. On other days the same dogs were taken out for a 4- to 6-mile brisk walk before the 2- to 4-hour recording period.

Records of the gastric hunger contractions were taken with the dog lying quietly in the lap of an assistant. Then the body of the dog was surrounded with an ice pack, or the dog placed directly on a slab of ice. After some training the dogs do not appear much disturbed by the ice pack or slab of ice. The ice pack was applied with the stomach quiescent as well as in hunger activity.

All of the above procedures were used on normal dogs and on dogs with the splanchnic nerves sectioned on both sides, in order

to have the tonus fibres of the vagi unopposed by the splanchnic inhibitory influence.

Experiments on Man.—The tests were made on the author, on Mr. V. (the gastric fistula case), and on three assistants (J. H. L., S. J. O., A. M. P.).

Records were taken of the gastric hunger and tonus contractions with the man standing or walking or running in place. Tests were also made after muscular exercise (playing tennis, walking 6 to 12 miles).

The influence of exposure to cold on the gastric hunger mechanism was tested in the following way. (1) While records of the gastric tonus and hunger contractions were being taken, the man, stripped of his clothes, was subjected to cold or warm showers for varying periods. The cold showers were at times sufficiently cold or prolonged to cause intense shivering. (2) The man stripped of his clothes in a cold room was covered up on a couch so as to feel comfortably warm. At the desired moment in the gastric activity, that is, during a period of quiescence or in the midst of a period of hunger contractions, the covers were removed and the cold air of the room set in motion by a fan placed close to the person. This brought on shivering in a few minutes. (3) The man arose at 7 A.M. and, without the usual cold bath and breakfast, proceeded to the laboratory and records of the gastric tonus and hunger contractions were taken from 8 to 12 A.M. These served as controls. On other days the man arose at 6 A.M., took a cold bath (this was prolonged until the discomfort became very severe), followed by a brisk walk, when records were taken from 8 to 12 A.M.

RESULTS OF EXPERIMENTS ON DOGS

1. *Effects of Running in Treadmill.*—The initial effect on gastric tonus and hunger contractions of running in the mill is always in the direction of inhibition—usually complete inhibition, and if the dog is started running in the midst of a period of gastric quiescence there is no evidence of increased gastric tonus or beginning hunger contractions. If the dog is made to run at high speed the inhibition persists during the entire period even

if the running is kept up for one or two hours. When the dogs ran at rather high speed for an hour or more the gastric inhibition usually persisted from 20 to 40 minutes after the dog stopped running. The return of gastric tonus and hunger contractions in such cases is very gradual. But usually when the gastric tonus finally recovered after a running period it was higher than before the dogs began to run. Thus a dog showing Type I and II hunger contractions when he started to run in the mill may show an increased tonus and Type III hunger contractions 30 minutes after he stopped running, while the running period itself was accompanied by complete gastric inhibition. If the dog runs only moderately fast in the mill the gastric tonus and hunger contractions reappear during the running period, or come on during the running, in case the dog is started when the empty stomach is quiescent.

These facts indicate that the carnivorous animal in pursuit of its prey must be urged on by something else than the pangs of hunger, as these are inhibited by the chase. Brisk walking or running leads also to inhibition of the digestion movements of the stomach, according to the observation of Cohn on dogs, Bender on man, and Scheunert on the horse.

2. *Effects of 4–6 Mile Walk.*—Eight tests (with a corresponding number of controls) on two dogs failed to show any marked effect of a 4–6 mile walk on the gastric hunger contractions either in the way of increase or decrease, the records being taken during the two hours following the walk. These walks certainly caused no depression of the dog's hunger contractions. But the dog that showed Type II contractions in the control usually showed Type II contractions after the walk with no definite increase either in rate or intensity. This should be noted, however, that after these walks both dogs showed greater restlessness than when taken from the kennels directly to the laboratory. They were not so easily quieted in the lap of the assistant. This rather restless condition of the dogs may have counteracted any augmentation of gastric hunger contractions due to the walk, as restlessness from any cause tends in the dog to inhibit the hunger contractions.

3. *The Effect of Intense Stimulation of the Cutaneous Nerve-endings for the Sensation of Cold.*—When a dog is lying quietly and comfortably in the lap of an assistant, surrounding the dog with an ice pack or placing him directly on a slab of ice leads to struggling and restlessness. After a number of repetitions of these procedures most dogs become so accustomed to it that they pay little or no attention to the change and show no restlessness or struggling. If the dog is disturbed or struggles when placed on the slab of ice or surrounded by an ice pack there always follows a temporary inhibition of gastric tonus and hunger contractions. But this does not indicate the initial or primary effect of stimulation of the cutaneous nerve-endings for cold, because the same type of inhibition is induced by restlessness or struggle for any cause. After the dog is trained for these procedures strong stimulation of the cutaneous nerve-endings for cold by the ice pack, by placing the dog on a slab of ice, or by turning on an electric fan in a cold room after uncovering the dog, has no immediate effect on the gastric tonus and hunger contractions. There is usually an increase in the intra-abdominal pressure owing to the increased tonus of the abdominal muscles. If the ice pack is applied during a period of gastric quiescence there is no immediate increase in gastric tonus or initiation of the hunger contractions, even though the dog starts to shiver violently in a few minutes. If the ice pack is applied during the hunger contractions, these contractions do not change appreciably either in rate or strength, at least for some time. This is true even when the dog shivers considerably. It would thus seem that the vagus centres governing the gastric tonus are not directly affected by even very strong stimulation of the cutaneous nerve-endings for cold.

In several instances the continued application of the ice pack (30 to 40 minutes) and in consequence continued shivering led to a gradually increased gastric tonus and the appearance of Type III hunger contractions. These may be due to changes in the blood as a result of increased oxidation, or they may appear from causes not connected with the stimulation of the cold nerve-endings. Such change in the hunger contractions is not infre-

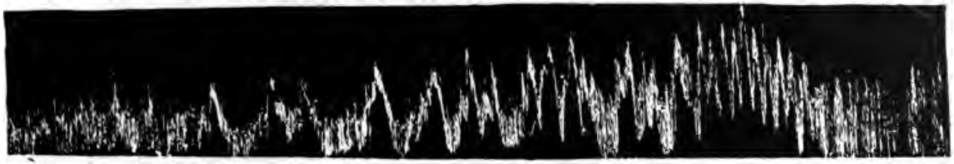

FIG. 1.—A period of gastric hunger contractions of a human infant, nine hours after birth, and before nursing.

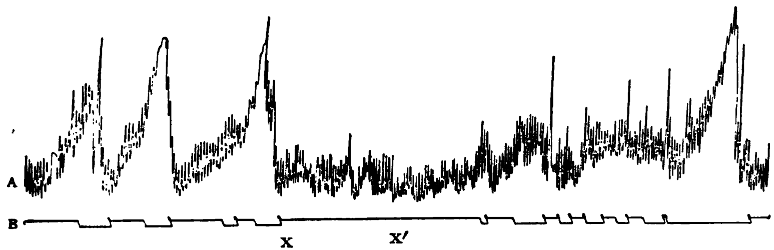

FIG. 2.—One-third the original size. A, stomach contractions; B, hunger signal; X-X′, chewing meat (palatable). Showing inhibition of the contractions of the empty stomach and the parallel cessation of the hunger pangs.

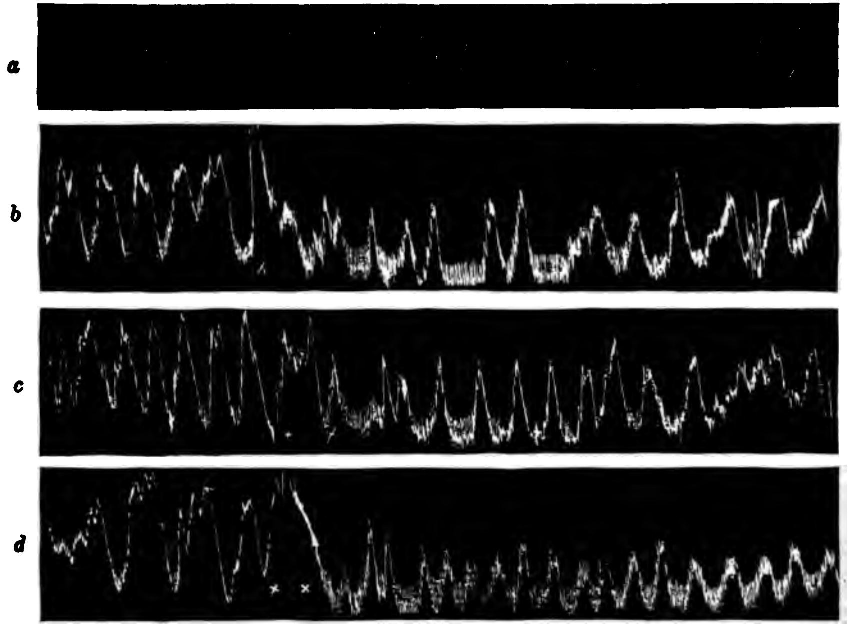

Fig. 3.—*a*, 10 c.c. of 0.25 per cent. hydrochloric acid put into the stomach during normal digestion peristalsis; *b*, 10 c.c. of 0.25 per cent. hydrochloric acid put into the stomach of a hungry rabbit; *c*, 10 c.c. of water put into the stomach of a hungry rabbit; *d*, 10 c.c. of 10 per cent. alcohol put into the stomach of a hungry rabbit. Note the inhibitory effect of these solutions on the hunger movements.

FIG. 4

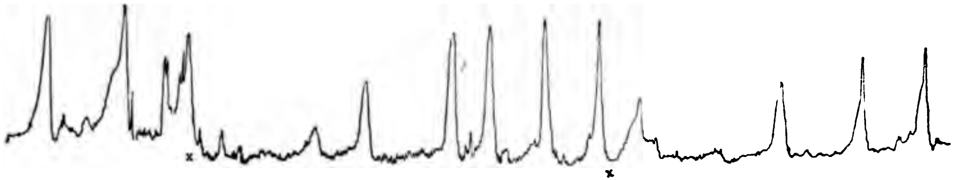

FIG. 5

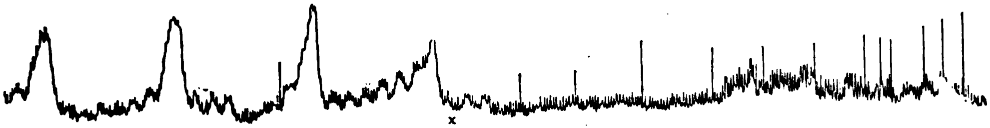

FIG. 6

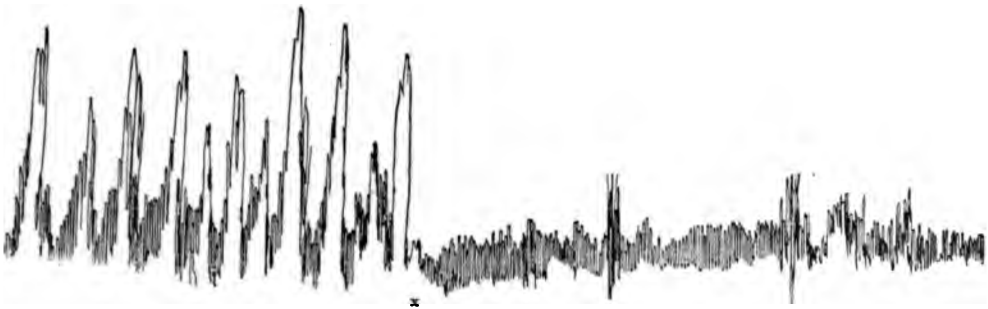

FIG. 4.—About two-thirds the original size. Record of contractions of the empty stomach of Mr. V. at X 100 c.c. cold water introduced directly into the stomach. Showing the temporary inhibition.

FIG. 5.—About one-half the original size. Record of contractions of the empty stomach of Mr. V. at X 25 c.c. of human gastric juice (V's own gastric juice, psychic secretion, secured two hours previously) introduced into the stomach. Showing the acid inhibition.

FIG. 6.—Records from the empty stomach of A. J. C. At X introduction of 15 c.c. warm water directly into the stomach. Showing the alcohol inhibition of the hunger contractions.

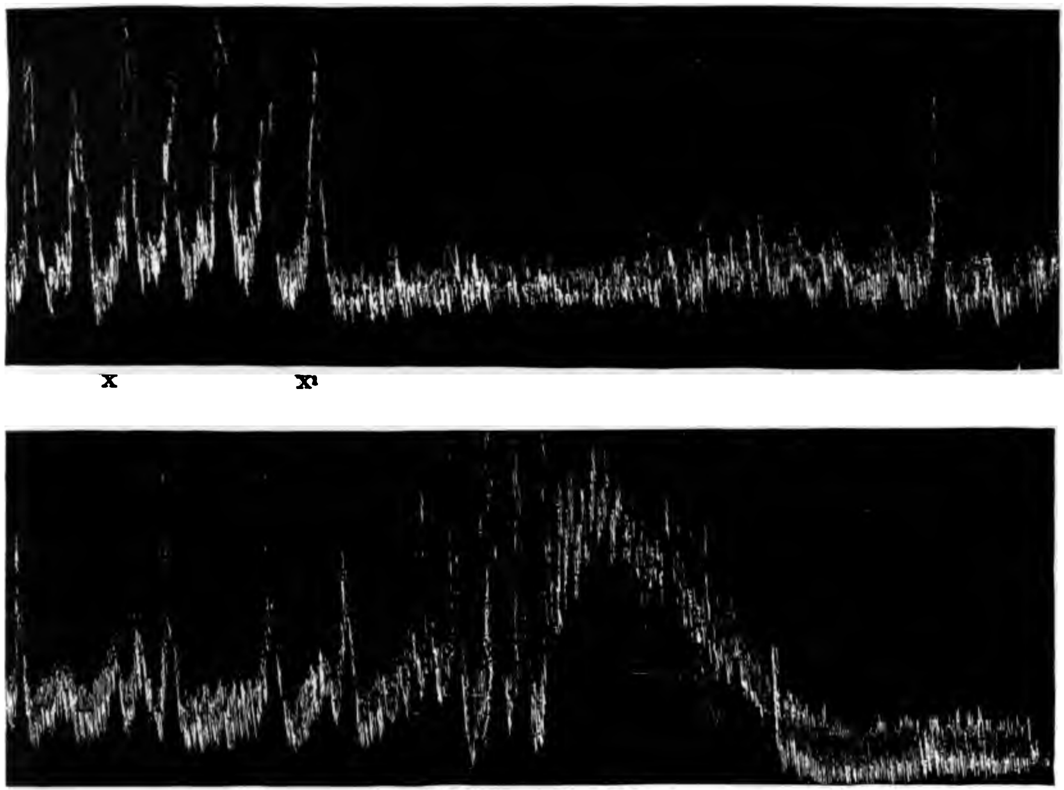

Fig. 7.—Record of the gastric hunger contraction, J. H. L. X, introduction of 6 c.c. tincture of condurango, showing no action on the gastric hunger mechanism. X′, few drops of same bitter put in mouth, showing complete and prolonged inhibition of the hunger contractions, with subsequent recovery and the completion of the hunger period in hunger tetanus typical for Mr. L.

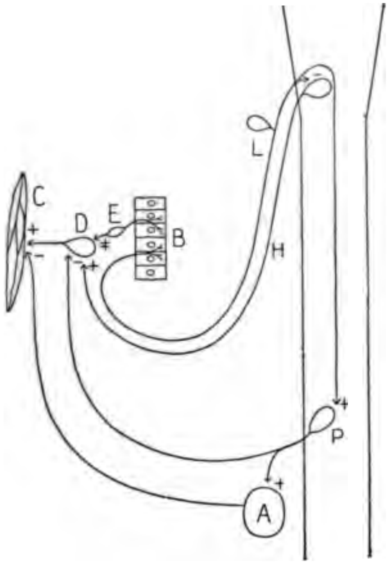

Fig. 8.—Diagram to represent the local and the long reflex mechanisms involved in the inhibition of the gastric tonus and the hunger contractions from stimulation of the gastric mucosa. A, adrenal gland; B, gastric mucosa; C, stomach musculature; D, Auerbach's plexus; E, local afferent neurones from the gastric mucosa to the Auerbach's plexus (these neurones are predominantly inhibitory); H, tonus or motor neurones to the stomach via the vagi, L, afferent neurones in the vagi from the gastric mucosa; P, neurones in the splanchnic nerves; + = stimulation; − = inhibition.

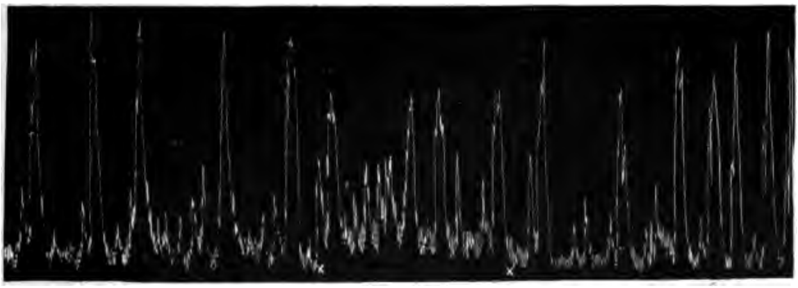

Fig. 9.—Record of gastric hunger contractions of A. J. C. X–X, intense stimulation of the cold nerve endings of the skin. Showing stimulation.

A. B.

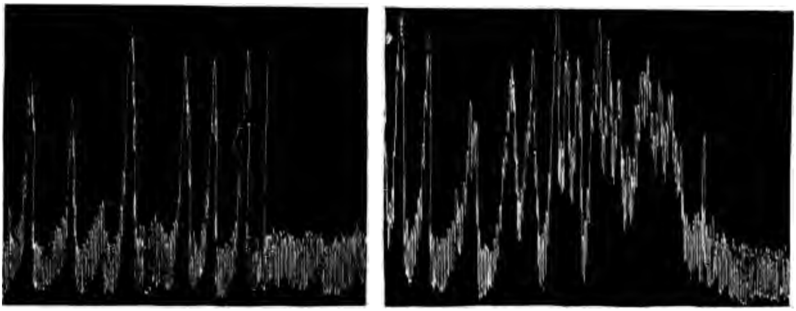

Fig. 10.—Record of the gastric hunger contractions of A. J. C. A, showing the usual ending of the hunger period; B, showing a hunger period ending in hunger tetanus a few hours after prolonged and intense stimulation of the cold nerve endings of the skin.

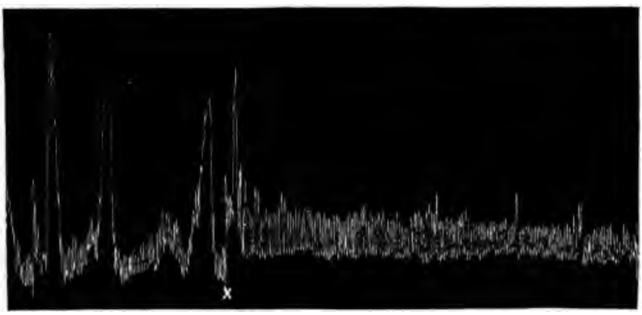

Fig. 11.—Record of the gastric hunger contractions (man), showing inhibition on strong constriction of abdominal belt (at X).

quent in dogs, even when they are lying undisturbed and comfortable in the lap of an assistant.

In two cases the Type III hunger contractions changed to Type II on removal of the ice pack (Fig. 2A). The change came on promptly on removing the ice pack. We are inclined to attribute this change to some shifting of the position of the stomach or shifting of the position of the balloon in the stomach as the result of the removal of the pressure of the ice pack over the abdomen, rather than a reflex effect.

It is conceivable that the stimulation of the cold nerve-endings in the skin does influence the vago-gastric tonus centres, but the stimulation acts equally on the gastric inhibitory mechanism via the splanchnic nerves so that the net result on the empty stomach is nil. This possibility is cleared up by the test on dogs with section of both splanchnic nerves. Tests were made on two dogs on which the operation had been performed. The results were practically identical with those on normal dogs. The ice pack neither decreased nor increased the gastric hunger contractions. It is, therefore, clear that the nervous impulses that give rise to the sensation of cold and induce increased neuromuscular tonus in general have no direct action on the vago-gastric tonus centres.

RESULTS ON MAN

1. *The Direct Effect of Muscular Exercise.*—Standing or walking in place has no effect on the gastric tonus or the hunger contractions. But running in place promptly inhibits the hunger contractions. The degree and duration of the inhibition is on the whole directly proportional to the speed of the running. In some cases walking seemed to prolong a hunger period without changing the rate or intensity of the individual contractions. In no case did walking or hunger induce hunger contractions in the quiescent stomach. The results on man are thus identical with the results on dogs. In both species rapid running is accompanied by inhibition of the gastric tonus and hunger contractions. In the case of the dog running in the treadmill, one cannot be sure that the exercise is strictly voluntary and enjoyable. The inhibition may therefore be due to certain emotional

states (anxiety, discomfort, mild anger, or fear). This possibility is eliminated by the tests on man. In the men the conditions of the emotions when running in place were not different from that when standing or walking in place. In no case was the running carried to the point of respiratory, cardiac, or muscular distress.

2. *The After-Effects of Muscular Exercise.*—Moderate exercise in the form of playing tennis or walking four to eight miles was taken in the afternoon. No supper was taken, and the motor condition of the empty stomach was recorded from 8 to 12 P.M. The tracings obtained on the day's specific exercise were taken to show on the whole greater gastric hunger activity than the control days. The periods of quiescence become shorter. This tends to make the gastric hunger contractions more or less continuous, and there appears to be some increase in the rate of the contractions. A typical experiment (S. J. O.) may be cited in the way of illustration.

Records of Control Day.—Lunch 1.30 P.M. No special exercise. No supper. Period of observation 8 to 12 P.M.

8 to 10 P.M. Stomach practically quiescent.

10 to 10.40. Strong hunger contractions, ending in tetanus.

10.40 to 11.35. Stomach quiescent.

11.35 to 12.05. Moderate hunger contractions ending in tetanus.

Record of Exercise Day.—Lunch 1.30 P.M. No supper, tennis 4 to 5 P.M.; walking 6 to 7 P.M. Period of observation 8 to 12 P.M.

8.15 to 9.50. Practically continuous hunger contraction ending in strong tetanus.

9.50 to 10.20. Stomach quiescent.

10.20 to 11.40. Strong hunger contractions ending in tetanus.

Total duration of hunger periods from 8 to 12 P.M.: Control day, 70 min.; exercise day, 190 min.

In some instance there was no marked difference between records of the control and the exercise days. This is to be expected, since the activity of the gastric hunger mechanism depends in part on factors not understood or controlled. Exercise that brings on a degree of fatigue bordering on exhaustion seems to depress the gastric hunger mechanism. But our experiments on this point are as yet too few to permit a final conclusion.

2. *The Direct Effect of Stimulation of the Cold Nerve-Endings of the Skin.*—The immediate effect of stimulation of the cold nerve-endings of the skin by ice pack, alcohol bath, cold shower bath, or cooled air is inhibition of the gastric tonus and hunger contractions, and the degree of inhibition is proportional to the intensity of the stimulation: In no instance did we observe an initial increase in gastric tonus and hunger contractions. When the stimulation is continued the inhibitory effects gradually diminish even though the man shivers intensely from the cold. In this way the gastric hunger contractions may return to their normal rate, intensity and regularity, while the man is shivering and jerking like a dog in mild parathyroid tetany. It may be noted in this connection that mild, and in some instances fairly severe, parathyroid tetany in dogs does not appreciably influence the gastric hunger contractions.

Intense stimulation of the heat nerve-endings of the skin (hot shower) produces practically the same initial inhibition as the corresponding stimulation of the cold nerve-endings.

While it is true that on prolonged stimulation of the cold nerve-endings of the skin during a period of gastric hunger contractions, the inhibitory effects gradually disappear so that the contractions reappear in their normal intensity, these contractions are always felt as weaker than the normal, or may not be felt at all. Evidently the intense sensation of cold dominates consciousness to the exclusion of the gastric hunger pangs.

It is well known that strong stimulation of the cold nerve-endings of the skin causes a reflex increase of tonus of the urinary bladder. In several instances we started these stomach tests on the men at a time when their bladder was known to contain 50 to 200 c.c. of urine. This permitted us to compare the reflex effect of cold on the stomach and bladder tonus without a balloon in the bladder. When the cold stimulation began during a period of gastric quiescence and was continued long to induce intense shivering, a strong desire to micturate soon developed, while there was no evidence of increased gastric tonus. Prolonged cold stimulation may produce so great tonus of the bladder that micturition cannot be inhibited voluntarily. The tonus centres of

the urinary bladder are, the vago-gastric tonus centres are not, directly influenced by cold stimulation of the skin.

When the cold nerve-endings of the skin are stimulated, as above, during a period of quiescence of the empty stomach, the stomach remains quiescent. If there is any change in the gastric tonus it is in the direction of inhibition. Nevertheless, this cold stimulation, if not sufficiently intense to be painful, seemed to induce a "sensation of emptiness" in the abdominal region, a sensation that seemed to be associated with appetite and desire for food. We record this with some hesitation, for this sensation of emptiness may be purely subjective (autosuggestion). It may also be due to the increased tonus of the abdominal muscles. In any event, this sensation is clearly different from the hunger pangs.

4. *The After-Effect of the Stimulation of the Cold Nerve-Endings of the Skin.*—All of the tests in this group were made on one man (A. J. C.). A prolonged cold bath 6 to 7 A.M. followed by a brisk walk nearly always resulted in increased hunger activity of the stomach as recorded for the period 8 to 12 A.M. The temperature of the water varied from 5° C. to 15° C. The subject remained in the water as long as was deemed safe (10 to 20 minutes), despite discomfort and pain. Water at this temperature soon brings on shivering, contracture and at times severe headache, and it requires much vigorous exercise to restore the feeling of warmth. Rubbing the skin (rough towel) seems to be of no aid.

Control Record.—No bath or breakfast. Observation period 8 to 12 A.M.
8.50 to 10 A.M., 26 fairly strong hunger contractions; no tetanus.
11 to 11.45, 22 fairly strong hunger contractions; no tetanus.
Gastric tonus on the average 5 cm. Bromoform.

Test Period.—6 to 6.15 A.M., cold bath (temp. of water 10° C.). No breakfast. Observation period 8 to 12 A.M.
8 to 9 A.M., 32 strong contractions; no hunger tetanus.
9.45 to 10.25, 23 fairly strong contractions; no hunger tetanus.
11.15 to 11.45, 19 strong contractions ending in hunger tetanus.
Gastric tonus on the average 8 cm. Bromoform.

Control Period.—48 hunger contractions; no hunger tetanus.
Test Period.—74 hunger contractions: hunger tetanus.

Under ordinary conditions the periods of gastric hunger contractions of the author do not end in tetanus, but the hunger tetanus appears after 3 to 4 days' complete starvation. Fifteen to thirty minutes' intense stimulation of the cold nerve-endings thus seem to bring about a condition similar to prolonged starvation. This is in harmony with the observation of Lusk that such stimulation quickly renders the liver free from glycogen. This effect of cold on the gastric hunger mechanism is obviously an indirect one, or through changes in the blood, and not a direct reflex from the skin.

Lusk has shown that intense cold leads to quicker and more complete oxidation of the body glycogen than prolonged starvation. And it is interesting to note that the same stimulus causes not only an increase in the *gastric hunger contractions,* but also an even greater increase in the subjective hunger and appetite sensations, probably owing to an increased excitability of the central nervous system. The increased desire to eat after a cold bath, in the case of the healthy individual, is a universal experience. We have investigated this matter in the case of young children, with whom habit and intelligence cannot be assigned as the cause for seeking food after a cold bath. It was found that young children react in the same way as adults.

While these observations include only two species (man, dog), it does not seem likely that the gastric vago-tonus mechanism will have different reflex associations in other animals. But this opinion should not stand in the way of actual investigation of the condition in other vertebrates as well as in the invertebrates. In man and in dog the situation appears to be this: The vagus motor nuclei in the medulla control, in part, the tonus and hunger contractions of the stomach. The tonus of the vagus nuclei, in turn, are controlled by the condition of the blood rather than by afferent nervous impulses, unless sensory impulses from the stomach musculature itself play such a rôle. This possibility is now being investigated.

VII. THE AFFERENT OR SENSORY PATHS OF THE HUNGER COMPLEX AND THE QUESTION OF THE CEREBRAL "HUNGER CENTRE"

1. *The Role of the Vagi.*—The vagi nerves are the main, if not the only afferent pathway for the gastric hunger impulses. If the contractions of the small intestine contribute to the hunger sensation, afferent hunger impulses may involve sympathetic and spinal nerves, but all sensory conduction from the stomach appear to be confined to the vagi, because no central reflexes of any kind can be evoked by stimulation of the stomach after section of all the vagi fibres to that organ (Miller). We need not here refer to the physiologists who have argued against the vagi nerves (and in favor of the splanchnic) as being concerned in hunger on the basis that animals will continue to eat after section of these nerves or after excision of the stomach, as it has not been shown that such animals eat because they feel hungry.

2. *The Primary Hunger Centre is Therefore the Sensory Nuclei of the Vagi Nerves in the Medulla (Fasciculus Solitarius).*—Some of the more direct hunger reflexes (such as salivation, vasomotor fluctuations, etc.) may be carried out via these medullary centres alone. Luciani assumes also spinal hunger centres analogous to these sensory vagi nuclei. There is no evidence that the processes of conscious hunger sensation can take place in the medullary nuclei.

3. *The Rôle of the Optic Thalami and the Mid-brain.*—The important facts in this connection are the hunger behavior of decerebrated animals (acephalic infants, dogs, pigeons, frogs). These animals minus the cerebral hemisphere, but with the thalamic region of the brain intact, exhibit practically all the hunger behavior of normal animals, except the intelligent search for and ingestion of the food. But even this statement requires limitation, for according to Ewald, decerebrated pigeons and frogs will finally eat spontaneously if kept in good condition for a sufficient time (months) after the operation.

Rogers has recently made the important observation that the hunger behavior of the decerebrated pigeon is completely abolished on removal of the optic thalami. It is thus clear that this

region contains important nuclei for the elaboration of the bodily responses to the hunger impulses from the stomach. Whether or not the processes of conscious hunger sensations are elaborated in the thalamus cannot be determined on experimental animals. L. R. Müller assumes that conscious hunger sensations are evoked in the mid-brain. We have seen that hunger is essentially pain, and some neurologists take the position that the sensation of pain is a thalamic rather than a cortical function. This view is supported by the extensive studies of Head and Holmes on the change in the pain sense in persons with thalamic lesions and intact cortex. The frequent occurrence of excessive hunger or polyphagia in persons with tumors of the pineal glands have by some (Schüller) been interpreted as due to a pressure stimulation on subcortical hunger centres. Whether or not the thalamic processes caused by the gastric hunger contractions are conscious or merely sub-conscious reflexes, or whether the nuclei concerned with these processes are identical with those involved in pain sensation, it is clear that the thalamus is a very important reflex and relay station for the afferent hunger impulses.

4. *Cortical Hunger Centres.*—Concerning these practically nothing is known. There can be little doubt that conscious hunger involves in some way cortical processes, and one might expect that the part of the cortex involved would be contiguous to that for the gustatory sense, the latter being placed in the hippocampal gyrus by the majority of neurologists and psychiatrists. Roux assumes that the cortical hunger centre is in the Rolandic area, thus supporting Bechterew, who locates the conscious taste processes in the region of the Rolandic area which innervates the muscles of mastication and deglutition.

To recapitulate: In the afferent phase of the hunger complex the facts clearly established are the rôle of the vagi, the sensory vagi nuclei in the medulla, and the great importance of the thalamus. The cortical factors in hunger are unknown and the same applies to the detailed rôles of the subcortical hunger centres in health and disease. This field of the physiology of hunger is therefore mainly "gaps and guesses." It remains for the *clinical investigator* to help correct the guesses, and fill up the gaps,

as very little can be done with these problems on animals below man, at least with the methods of to-day.

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THE RESPIRATION CALORIMETER IN CLINICAL MEDICINE *

DOCTOR EUGENE F. DU BOIS
Russell Sage Institute of Pathology in affiliation with the Second Medical Division, Bellevue Hospital, New York

IN THESE days when the housewives speak in terms of calories and the newspapers deal with vitamines it may be well for clinicians to examine once more the fundamental principles of nutrition. Patients no longer demand medicines, but they do expect diets, and if they are at all up with the magazine literature, they are better informed on the subject than the text-books which most of us studied in the medical school. The diets which patients receive in many of our hospitals were laboriously compiled several generations ago and modified from time to time by hospital dietitians. Foods were classified according to their solidity as administered, regardless of what happened in the stomach. Details were left to the head nurse and sometimes to their probationer, and while the patient always had the veto power because he could vomit, he was seldom able to initiate legislation.

The science of nutrition in disease must be founded upon experiments in calorimetry and the respiratory metabolism. It may be well at this point to state just what is meant by the term metabolism. The word in itself indicates the transformation of matter and refers to the breaking down of foods, to their absorption and to the building up of body tissues. The term also refers to the oxidation of foodstuffs and of body tissues, with the consequent liberation of warmth and energy. Analysis of food, urine and blood gives an incomplete picture, and it is only through a study of the gaseous exchanges in the tissues that we can study the total metabolism. There are many phrases which

* Delivered November 27, 1915.

have the same meaning as total metabolism. Frequently it is spoken of as *the* metabolism. Sometimes it is called the total energy requirement, total food requirement or heat production, total oxidative processes or total gaseous exchange.

In the years between 1892 and 1908 there was no work being done on this subject in our American hospitals, although Atwater and his successor Benedict, in Middletown, were making classical experiments on normal men, and Lusk, here in New York, was making similar experiments on dogs. During this same period in Germany the respiratory metabolism was being studied in a dozen clinics and the investigators were hoping that at some time means would be found to apply the apparatus of Atwater and Benedict to the study of disease, since this alone would solve certain problems. About seven years ago, Benedict and Joslin,[1, 2, 3] in Boston, began to study the respiratory metabolism of diabetes, and four years ago investigation of the gaseous exchanges of typhoid patients was begun in Bellevue Hospital.[4] Shortly afterward the directors of the Russell Sage Institute of Pathology decided to turn over their funds to Prof. Graham Lusk for a period of five years in order that a calorimeter might be built and maintained in Bellevue.

This respiration apparatus is perhaps the most complicated piece of machinery used in modern medicine, but the principles involved in its construction are simple. The description would seem to be a mass of technical details were it not for the fact that it illustrates beautifully the manner in which medicine is founded on physiology and physiology on physics and chemistry.

The first Atwater-Rosa[5] calorimeter in Middletown was a large chamber in which a man could lie in bed, stand up or ride a stationary bicycle. This apparatus is now in Washington, under the charge of Langworthy and Milner,[6] who have made many improvements. Atwater's work, however, has been chiefly continued by Dr. F. G. Benedict,[7, 8] of the Nutrition Laboratory in Boston, where great advances in the science of calorimetry have been made. Dr. H. B. Williams,[9] of New York, who constructed the small and very accurate calorimeter for Lusk at the Cornell Medical College, has added many ingenious devices. The Sage Calorim-

eter was finished by Mr. J. A. Riche and Mr. G. F. Soderstrom,[10] in the early part of 1913. It has all the modern improvements and is especially adapted for clinical work where observations must be short. Fortunately it is situated in a room near the medical wards of the second division of Bellevue. It is next door to a small diet kitchen and metabolism ward, where three specially trained nurses weigh out the food and collect the specimens.

The calorimeter itself consists of a copper box about the size of the lower berth of a sleeping car. It contains a comfortable bed and is provided with two windows, a shelf, a telephone, a fan, a light, and a Bowles stethoscope for counting the pulse. The ordinary experiment takes about as long as a trip from New York to New London, and there is an agreeable absence of cinders. Patients, as a rule, doze from time to time or else try to work out some scheme by which they can amuse themselves without moving. After three or four hours they are rather bored by the quiet, and the observations are not prolonged beyond this time. They are allowed to turn over in bed once or twice an hour, but reading and telephoning are discouraged, since these increase the metabolism. The air in the box is fresh and pure, the patient suffers no discomfort, and objections to the procedure are very infrequent. Most of the patients are only too glad of the extra attention, and they insist that the calorimeter has a marked therapeutic value.

The apparatus has two distinct functions: (1) the physical measurement of the heat production of an individual by the method of direct calorimetry; (2) the chemical measurement of his gaseous exchanges and the calculation of the heat by the method of indirect calorimetry. Both depend on the fact that the apparatus is a closed circuit, absolutely shut off from the surrounding atmosphere in such a manner that everything eliminated by the subject is caught and measured.

None of the heat radiated from the man's body can travel through the insulating walls of the calorimeter. All is removed and measured in a stream of cold water flowing through a pipe in the top of the box. It is necessary to determine also the heat dissipated from the body in the vaporization of moisture from skin

and lungs. A man loses about one-quarter of the total heat in this manner, and the 20 to 30 grams of water evaporated each hour are caught in a sulphuric acid bottle in the ventilating current. The grams of water multiplied by the factor for the latent heat of vaporization gives us the calories so eliminated. These measurements are very accurate, but it is difficult to determine how much heat a man stores in his body when his temperature rises or how much he releases from his body when his temperature falls. The rectal thermometer does not always tell us of the changes in the average body temperature, and an error of 0.1° C. may cause an error of 5 or 6 calories. It is for this reason that direct calorimetry is difficult when the fluctuations of temperature are great. When short periods are employed we therefore place our chief reliance on the method of indirect calorimetry.

This second method is entirely chemical; and, since it involves a different set of measurements, it serves as an excellent check on the accuracy of the results. The calorimeter is air-tight, and is connected with a series of absorbing bottles by means of a closed circuit or pipes. Air is drawn from the foot of the box by a rotary blower, passed through a bottle of strong sulphuric acid to remove the water vapor and then through weighed bottles of soda-lime and sulphuric acid, which catch the carbon dioxide. By noting the change in weights of these bottles the amounts of water and carbon dioxide removed may be determined. The air thus purified is returned to the box and is used again and again. Meanwhile the subject is consuming oxygen, and this would decrease the volume of gases within the box were it not for the fact that oxygen is automatically admitted from a weighed bomb to compensate exactly for the amount consumed. If we divide the liters of carbon dioxide produced by the liters of oxygen consumed we obtain the respiratory quotient. Knowing this and the amount of nitrogen eliminated in the urine it is possible to calculate the grams of protein, fat, and carbohydrate metabolized each hour. From their well-known heat values we can reckon out the calories produced. With the exception of a portion of the protein molecule each foodstuff is oxidized to the same end-products and with the same liberation of heat in the body as in the Liebig combustion fur-

nace or the bomb calorimeter. The process is slower but just as complete, and there is no loss of energy.

The experimental procedure is not very complicated. A patient is given his ordinary supper in the evening. The next morning his breakfast is withheld and he is put in the calorimeter at about ten o'clock. By eleven o'clock the machine is brought into thermal equilibrium and the experiment is started. At the end of each hour the ventilating current is switched to a new set of absorbing bottles, and by one or two o'clock the observation is over. Calculations consume an hour or so more, and as soon as the urinary nitrogen is determined we have the following information in regard to the patient in hourly periods: consumption of oxygen; output of carbon dioxide; output of water and the total calories; percentage of calories furnished by protein, by fat, by carbohydrate; percentage of heat lost by vaporization, by radiation, and conduction and by storage in or loss from the body; and finally, total metabolism and its relation to the average normal figure, this total metabolism being measured by two independent methods. As may be supposed, these activities keep the three observers fairly busy, since they involve about 40 weighings, 500 temperature readings, and the writing of over 4000 figures.

The accuracy of the calorimeter is tested at regular intervals by burning known amounts of alcohol and comparing the actual findings with the theoretical. In 9 such tests the average error for heat production was 0.9 per cent., for oxygen 1.2 per cent., for carbon dioxide 0.8 per cent. The total errors are even smaller.

It is hardly necessary to point out that the results obtained with the calorimeter are due to the team work of the whole staff, including the nurses who administer the diets and the chemists who analyze food, blood, urine, etc. Those chiefly responsible for the work here presented are: Dr. Lusk, the scientific director; Mr. Gephart, Dr. Meyer, Mr. Soderstrom, Mr. Harries, Miss Magill, all of the Sage staff, and our associates, Dr. Warren Coleman, Dr. F. M. Allen, and Dr. F. W. Peabody.

There have been many types of apparatus used in the study of the respiratory metabolism in disease.[11] Unfortunately time does not permit of their description, but one cannot help mentioning

by name the Pettenkofer-Voit chamber, the Zuntz-Geppert apparatus, the Benedict Unit,[12, 13] and the Paschutin calorimeter.

The main object of all investigators has been to determine the heat production of the patient while at complete rest fourteen hours or more after the last meal. This is the so-called basal metabolism, and is of interest only when compared with the figures obtained on normal individuals. Since it is impossible to measure the metabolism of many of our patients when they are entirely recovered, it is necessary to calculate what the man's metabolism would be were he normal. Here lies the most difficult problem. Controversies have raged more fiercely about the normal controls than about the pathological cases. It has been said that a man is as old as his arteries. It may also be said that a piece of research work is as good as its normal controls.

The normal controls used by investigators up to the last few years often showed a variation of 50 per cent. above or below the average. It was discouraging to run the calorimeter within an error of 1 or 2 per cent. and then compare the results with such an uncertain figure. Now this variation in the normals was largely due to the manner in which the results were expressed. If we leave the calculations, as was previously done, in the stage of cubic centimetres of carbon dioxide per kilogram of body weight there might be a large apparent variation between two men whose heat production is identical. Part of this error is eliminated if we express the results in calories, and still more eliminated if we base our calculations on surface area rather than body weight. A large man, of course, gives off more heat than a small man, but for each kilogram of body weight the small man has the higher metabolism. Rubner[14] demonstrated many years ago that the metabolism is proportional to the surface area of the body and that for each square metre of skin large men, small men, dogs, horses, and mice have about the same heat production. Just why this should be we do not know. It reminds us at once of Newton's law that the cooling of bodies is proportional to their surface area, but the metabolism does not follow this law when the external temperature is raised or lowered.

There are several formulas which allow us to calculate the

surface area of a man. Meeh's [15] formula, which is proportional to the two-third power of the weight, has been the standard for over a generation, and up to the last year entered into almost all the calculations. One divided the calories produced per hour by the square metres of surface area and obtained the calories per square metre per hour. Very recently the accuracy of Meeh's formula was investigated by the Sage staff. Mr. Delafield Du Bois [16] devised a method of measuring the skin area which has been applied to ten individuals of every conceivable shape. He found an average plus error of 16 per cent. in Meeh's formula and an error of 36 per cent. in fat subjects. To correct these the so-called "linear formula" was devised and also a simpler formula based on height and weight.[17]

It is interesting to group the normal controls studied in the Sage calorimeter [18] with the large number of healthy men and women studied by Benedict, Emmes, Roth and Smith in Boston,[19, 20, 21, 22, 23] and also by Palmer, Means and Gamble.[25] If we plot the men under fifty years of age according to calories per square metre of surface area we find that all are within 15 per cent. of the averages and 86 per cent. are within 10 per cent. of the average. According to Meeh's formula thin people have an increased metabolism and fat people a very low metabolism. Using the more accurate linear formula, Means [24, 25, 26] has found the metabolism in most cases of obesity to be within normal limits, and if we recalculate the figures for groups of thin men and fat men according to their true surface area the results in the two groups are almost identical.

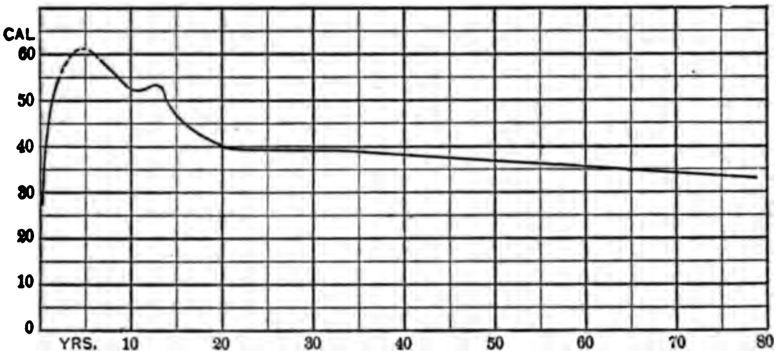
We can therefore feel certain that with men between the ages of twenty and fifty the metabolism of each individual is proportional to his surface area whether he be short or tall, fat or thin. We can compare all our pathological cases with the normal average basal metabolism of 34.7 calories per hour per square metre of surface according to Meeh's formula, or, better still, 39.7 per square metre according to the new so-called linear formula. The normal variation from this average is plus or minus 10 per cent. and a few apparently normal men show a variation of 15 per cent. We cannot consider the metabolism of an individual patient to be

abnormal if within these limits, but if a group of patients gives an average of more than 10 per cent. from the normal base line we can feel certain that there is a pathological change in the heat production in that particular disease.

Comparisons of different persons can be made only by determining the basal metabolism. There are many factors which cause certain groups of normal individuals to depart materially from our mean figure of 39.7 calories per square metre per hour. Women give an average about 6 per cent. below this,[22] athletes are about the same distance above, as has been shown by Benedict and Smith.[21] Age causes a marked difference which will be discussed later. The metabolism seems to be higher in the afternoon than in the morning, and during profound sleep there is a drop of 5 to 20 per cent. The metabolism is 5 to 10 per cent. higher sitting upright in a chair than when lying down, but it has recently been found that the metabolism of subjects propped up comfortably in a steamer chair is a little lower than when they are lying flat in bed.[27] This throws some light on one of the many causes of the assumption of the orthopnœic position by patients. It lends scientific support to the empirical observation that a lazy man is happiest with his chair tilted back and his feet on the desk.

As I have said, the basal metabolism is measured fourteen hours or more after the last meal because food stimulates the metabolism. This is due to the so-called specific dynamic action of foods, and is greatest in the case of protein and least in the case of carbohydrate. The phenomena, first discovered by Rubner,[28] have been studied in great detail by Lusk,[29] using the dog calorimeter at the Cornell Medical College. He has found that the specific dynamic action of protein is due to the stimulus of the metabolism products of amino-acids acting upon cell protoplasm, and that although the preliminary cleavage products of fat and carbohydrate do not appear to be direct stimuli, yet when they are present in excess in the fluid bathing the cells, the heat production is increased. With the normal controls in the Sage calorimeter [18] a standard protein meal containing 10 grams of nitrogen causes an average increase of 12 per cent. in the five

to six hours after its ingestion, and 100 grams of dextrose causes an increase of 9 per cent. These same meals have been used to test the specific dynamic action of foods in typhoid fever and exophthalmic goitre.



The curve showing the effect of age on heat production is of special interest. Magnus-Levy and Falk [30] demonstrated that the metabolism was high in childhood and low in old age, and no one will doubt this who has compared the amounts of food consumed by children and by their grandparents. We have now at our command a large number of observations made at all ages except the period between the second and sixth years. The reason for this gap is apparent. The shortest experimental period is ten minutes, and normal children of this age are never quiet for this length of time. The metabolism of infants has been studied by Howland,[31] Benedict and Talbot,[32, 33, 34] by Murlin and Hoobler,[35] Bailey and Murlin [36] and others. Eight boy scouts, not long ago, were studied in the Sage calorimeter [37] and made to keep quiet by a system of fines and rewards. If we tabulate these with all the other children and adults we can trace a curve which shows the heat production per square metre of surface at different ages, and compare it with the average adult figure. During the first few weeks of life the metabolism is very low, after which it rises rapidly until at the end of the first year it is 50 per cent. above the adult level. The curve reaches its acme in the almost unex-

plored period between two and six years and then falls fairly rapidly until the age of twenty years. After this the decrease is extremely gradual.

Just why newborn infants should have such a low metabolism we do not know. It is conceivable that the high metabolism of older children is in part due to the relatively large size of the head, body, and especially the liver. Men with their legs cut off resemble babies in the relative proportions of body and extremities. Two such individuals studied in the calorimeter have been found to have a normal metabolism. This would not explain the high metabolism of the boy scouts who have reached the adult proportions although they have not attained adult size. Theorists might go so far as to say that the increased heat production proves in itself an increased thyroid activity in childhood. It is safer for several reasons to ascribe it to some unknown stimulus associated with growth. For the level of metabolism falls as the rate of growth decreases, but rises again in the case of the boy scouts just before the onset of puberty, a period when the rate of growth is once more accelerated. Again, in adult life the nearest approach to the growth of childhood is found in convalescence from acute infectious diseases, and there is a distinct rise in metabolism in the convalescence from typhoid fever.[4, 38]

METABOLISM IN DISEASE

The most striking disease from the standpoint of changes in the total metabolism is exophthalmic goitre, and clinicians who pay no attention to the gaseous exchanges neglect the most important phase of this malady. Friedrich Müller first called attention to the high metabolism in Basedow's disease by noting the nitrogen and weight losses of patients taking enough food to maintain normal subjects in nutritive equilibrium. Magnus-Levy[40, 41] found the gaseous exchanges very high in Graves's disease and very low in myxœdema.[42] His results have been amply confirmed by Stüve,[43] Hirschlaff,[44] Salomon[45] and others, and by those of us working with the Sage calorimeter.[46] If we group 33 cases of hyperthyroidism studied by other investigators with the 11 studied in New York, we find that the increase in

metabolism is strictly proportional to the severity of the clinical symptoms. One fatal case of Hirschlaff's in the last two weeks of life showed an increase of 120 per cent. above the average normal and two severe but non-fatal cases at Bellevue have given results which are 100 per cent. above the normal. In general, it may be said that very severe cases show an increase of 75 per cent. or more, severe cases 50 per cent. or more, moderately severe and mild cases less than 50 per cent., while a few mild, atypical or operated cases may be within normal limits.

This increase in metabolism is the most striking effect of thyroid activity, and it is equalled in no other disease. In cretinism and myxœdema the metabolism may be 25 to 50 per cent. below the normal level. Thyroid extract raises the metabolism in these conditions and has a similar though less constant effect on normal men and obesity patients. No other glandular extract yet examined has this property in any significant degree. Many of the symptoms of hyperthyroidism are secondary to the abnormal heat production. The flushed, warm skin and the sweating are physiological methods of dissipating extra heat. The increased appetite and the loss of weight that sometimes occurs in spite of it are the results of increased calorific requirements. Part of the tachycardia is certainly due to the greater demands for oxygen.

Most of the investigators who have studied the gaseous exchanges in this disease have used the level of the metabolism as an index of the effect of treatment. This procedure is perfectly logical, and it is particularly desirable to have some purely objective guide to therapeusis in a disease where there is so much psychotherapy on the part of the physician and patient. Previous observers have found no change in the metabolism after the administration of rhadogen,[45] Röntgen rays,[47] and the serum of thyroidectomized animals,[45] but have established a prompt drop after partial thyroidectomy.[48] In the cases observed at Bellevue a fall of 10 or 15 per cent. has resulted from rest alone, and none of the other therapeutic measures tried gave any better results. These included Beebe's serum, ergotin and quinin hydrobromate, and finally thyroid residue. But since the two former methods

were tried on only one patient it is not fair to condemn them. With several patients the thyroid arteries were ligated, and it was found that this procedure caused a sharp rise in the metabolism which fell subsequently to its former level. The operative procedure, although very conservatively done under local anæsthesia, increases temporarily the activity of the gland. There is as yet no proof that any form of medical treatment or ligation of arteries causes a greater reduction in metabolism than rest in bed. Several important facts have been brought out in regard to the administration of food to Basedow patients. They need large amounts of food with an abundant, though not excessive, protein ration. Von Noorden [49] has warned us against the use of large amounts of protein and especially meat, assuming that it causes a greater rise in heat production in sickness than in health. Pribram and Porges [47] found that after a diet with large amounts of meat the metabolism was slightly increased, but not more so than in normal people. Undeutsch [48] found that the protein of meat causes less stimulation than vegetable proteins. With the Sage calorimeter it has been found that the specific dynamic action of protein and carbohydrate with exophthalmic goitre patients is not appreciably different from the normal, and that there is no significant difference between the effects of meat and the same amount of protein in milk and eggs. It has taken a large amount of work to disprove the hypothesis of a prominent specialist. Still, it is a great relief to know that we need not worry our patients with restrictions on the kinds of proteins they take. The protein ration should contain about 12 to 15 grams of nitrogen a day, which is the amount ordinarily consumed.

The carbohydrates are readily metabolized in spite of the moderate glycosuria found in severe cases. Except in the true cases of diabetes with goitre it is an abnormality of mobilization rather than utilization. One of the severe cases at Bellevue Hospital was able to derive 90 per cent. of his calories from the oxidation of carbohydrates in spite of a marked glycosuria. A severe case of diabetes could not have derived 10 per cent. of his calories from this source, and even a mild case would scarcely have reached the figure of 50 per cent. Goitre patients use up

carbohydrates rapidly, and fourteen hours after their last meal are maintaining themselves almost entirely on fat and protein.

In regard to the medical treatment of Graves's disease the calorimeter may be a therapeutic Nihilist, but it is a dietetic enthusiast, and it is also a strong supporter of the belief that mental and physical rest are essential in the treatment of severe cases.

Diabetes Mellitus.—The Harvey Society is fortunate in having, almost every year, a lecture devoted exclusively to the fascinating subject of diabetes mellitus. The respiratory metabolism was discussed last year in a masterly fashion by Joslin,[50] who, with Benedict, in Boston,[1, 2, 3] has done an enormous amount of work on this subject. During the past year, working in coöperation with Dr. Allen,[51] of the Rockefeller Hospital, it has been possible to study in the greatest detail one particularly severe case of diabetes snatched from coma by the Allen fasting treatment. This case and several others also studied have given such striking confirmation to the views held by Lusk,[52, 53, 54, 55] for many years, that it is impossible to pass the subject by. Lusk was the first to maintain that a patient who was completely diabetic would excrete not only all the ingested carbohydrate but would also turn about half of the protein molecule into sugar and excrete it in the urine. Under these conditions the excess of excreted sugar over ingested would be about three and a half times the nitrogen in the urine: in other words, the D:N ratio would be 3.65. Under these circumstances the respiratory quotient would be depressed below that of fat by the incomplete combustion of protein instead of being raised by protein as with normal people. The lowest possible quotient in health is 0.72, and in complete diabetes with the D:N ratio 3.65, Lusk has calculated that the quotient might be depressed to 0.69. One of the severe cases above mentioned with the D:N of 3.5 showed a quotient of 0.697, another with the D:N of 3.1 gave a quotient of 0.692. There was absolutely no evidence of any formation of sugar from fat. The D:N ratio if determined under the proper precautions is certainly the best guide as to the severity of the case.

The effect of the Allen [56] fasting treatment in the severe case most completely studied was to cause a gradual rise in the quotient as the D : N ratio fell and the glycosuria cleared up. After the fast the curious phenomenon mentioned by Joslin [50] was noted. There was a rise in quotient indicating the combustion of carbohydrate from some unknown source. This may be stored glycogen, excess of sugar in blood and tissues, or perhaps it may be due to the oxidation of acetone bodies.

Another effect of the fast was the marked fall in total metabolism. The patient was brought to a condition where his low food requirement could be met by his improved though still damaged metabolic functions. The metabolism was reduced to a point even lower than that reached by normal men fasting a long time.

This brings us to the question of the total metabolism in diabetes, still the subject of controversy. The older investigations by Pettenkofer and Voit,[57] Nehring and Schmoll,[58] Magnus-Levy,[59] Du Bois and Veeder [60] and others indicated only a slight increase in total metabolism. More recently Benedict and Joslin,[1, 2, 3] who have studied a large number of cases, have maintained that the metabolism averages about 15 per cent. above the normal in severe diabetes, and Rolly [61] and Leimdörfer [62] reached the same conclusion. Lusk [63] has gone over the results obtained by Benedict and Joslin, using different normal controls for the purposes of comparison, and has concluded that the metabolism is but slightly increased. An interesting light has been thrown on this subject by the new method of calculating the surface area. It was noted in one severe case of diabetes, studied before the fast at Bellevue Hospital, that the metabolism was 2 per cent. above the average figure according to Meeh's formula, but 8 per cent. below according to the true surface area. Patients with severe diabetes are usually very thin, and in such cases comparisons based on Meeh's formula are untrustworthy. If we recalculate the severe cases of Benedict and Joslin by means of the new height-weight formula we find that the average is about 3 per cent. above the standard figure of 39.7 calories per square metre per hour. The cases studied at Bellevue were all below

the normal, but some of them were low on account of fasting. (One patient very recently studied showed a slight increase in metabolism for a few days. His acidosis and also his nitrogen elimination were unusually high.)

Typhoid Fever.—Typhoid lends itself particularly well to a study of the changes in metabolism which occur in fever. It is more uniform in its course than most fevers, is protracted, is often severe, and is with us every autumn. There are several factors at work in a typhoid patient: (1) toxæmia, (2) fever, and (3) more or less starvation. Since the introduction of the Shaffer-Coleman [65] high calory diet this last factor has almost disappeared, leaving a much more satisfactory disease for the patient and for the scientific investigator. The work done on the nitrogen metabolism has been voluminous, and the controversy concerning the toxic destruction of protein is still violent. The respiratory metabolism has been studied by many investigators, notably Kraus,[66] Svenson,[67] Grafe,[68] and Rolly.[69] At Bellevue Hospital 134 observations with the small Benedict apparatus have been made by Coleman and the writer and 61 with the Sage calorimeter.

The total metabolism in typhoid fever shows an increase which is roughly proportional to the rise in temperature. At the height of the fever it averages about 40 per cent. above the normal, but in some cases may be more than 50 per cent. above. Patients who are liberally nourished with the high calory diet of Coleman and Shaffer do not have a greater heat production than patients on low diets. The Sage calorimeter has shown that the specific dynamic action of protein and glucose is much smaller than normal, and in fact almost absent in typhoid fever. The practical application of all this is that typhoid patients need more food than normal men under similar conditions, and that food even in large amounts is well absorbed and does not increase the heat production, as was previously feared. Typhoid patients like all others with high metabolism use up their carbohydrate food and glycogen stores rapidly, and this type of food should be given in large amounts if we do not want the patients to subsist on protein and fat obtained chiefly from their own tissues.

The actual heat production of most of the typhoid patients is between 2000 and 3000 calories a day. Such amounts, as a rule, can be readily administered, and to me personally it hardly seems necessary to give more than 3000 calories a day unless the patient wants it. Dr. Coleman, whose experience is much greater than mine, believes that patients do best on larger amounts, since these alone will prevent the loss of body protein.

A series of careful experiments has been made in which the nitrogen and respiratory metabolism was studied at the same time. Now a normal man can be maintained in nitrogen and weight equilibrium if given a moderate amount of protein and enough calories to cover the heat production as calculated from the basal metabolism, with allowance for food stimulation and muscular activity. It has been possible by means of the Sage calorimeter to determine the actual heat production of a series of typhoid patients. In the adjoining metabolism ward the head nurse, Miss Magill, and her assistants have skilfully administered food in sufficient amount amply to cover the caloric output. Yet the patients have shown distinctly negative nitrogen balances during the febrile period, and in one case for several days after the fever had ended. This is conclusive proof that there is an abnormal destruction of protein in typhoid fever, and the evidence indicates that this is due chiefly to the toxins of the disease. Shaffer and Coleman were able to demonstrate that this toxic destruction could be prevented or masked by the administration of very large amounts of food, particularly carbohydrates. Kocher[70] has recently shown that in fever even with large amounts of food the nitrogen excretion can never be reduced to the low point of 3 to 4 grams a day readily obtained in health.

In the early days of convalescence from typhoid the metabolism is slightly below the normal, then it rises to 15 to 20 per cent. above, apparently as the result of the large amounts of food and the stimulus of growth. In convalescence the specific dynamic action of food is either normal or else greater than normal. We do not care if the heat production at this period be increased and there is no contra-indication from the metabolic standpoint to liberal feeding in convalescence.

The problem of the mechanism of the rise and fall in body temperature in fever has been the subject of much experimentation, and the question can be settled only by a respiration calorimeter. For reasons that cannot be discussed in a short paper the technic is difficult, and there are only 11 of the calorimeter experiments in typhoid which meet the rigid requirements. These indicate that a rising temperature is accompanied by an increasing heat production which outweighs a slightly increasing heat elimination. With a falling temperature the production remains fairly level while the elimination is increased.

Anæmias.—The question of the oxidative processes in cases of severe anæmia is of particular interest. When the hæmoglobin content of the blood is greatly reduced it is difficult to see how the tissues can be supplied with enough oxygen, and yet every clinician has seen patients with 20 to 30 per cent. of the normal percentage of hæmoglobin who are not dyspnœic. How is this possible? The blood volume is not increased enough to account for the compensation, the plasma can carry only small amounts of oxygen, the hæmoglobin in anæmia does not possess an abnormal power to combine with extra amounts of oxygen. Some experimenters have believed that the oxygen requirement in such patients was lower than normal.

The respiratory metabolism in various types of anæmia has been studied by Magnus-Levy,[71] Kraus,[72] Bohland,[73] Thiele and Nehring,[74] Grafe,[75] and six cases of pernicious anæmia have been observed in the Sage calorimeter by Meyer and the writer.[76] In few cases examined by investigators I have named was the metabolism below normal. In lymphatic leukæmia it was often 50 per cent. above normal, perhaps on account of the abnormal oxidative activity of the white blood cells, perhaps on account of lactic acid formation. In two severe cases of pernicious anæmia at Bellevue Hospital with 20 per cent. hæmoglobin the metabolism was 14 and 39 per cent. above normal.

The chief compensatory factor in such patients would seem to be an increased cardiac output per minute. It appears also that the blood is more completely robbed of its oxygen in its passage through the capillaries.

Cardiorenal Disease.—The previous works on the gaseous exchanges in cardiac and renal disease have not been extensive and have been marred by respiratory quotients which are so low that it has been necessary for the investigators to assume abnormal processes. Peabody, Meyer and Du Bois [77] have studied 16 patients in the calorimeter, a number which is somewhat small when we consider the variations present in these diseases. In general it may be said that mild cases of nephritis and compensated cardiac patients are within normal limits, as are a few severe cases of both diseases. An increase in heat production amounting to 30 to 40 per cent. is found in most of the very dyspnœic patients. The cause of the rise may be in part the increased work of respiration and increased labor of the heart, but the chief factors are yet to be discovered.

There is no evidence of profound changes in the intermediary metabolism in cardiac and nephritic dyspnœa. Not a single abnormal quotient has been found by the calorimeter. We must remember that the patients with high metabolism use up more food than normal men, and with ordinary diet will suffer from partial starvation. If not given large amounts of carbohydrate in the food they will subsist chiefly on fat and protein derived largely from their own tissues. It is worth while to remember these things when we order the restricted diets which seem necessary in many cases of cardiac and renal disease.

Miscellaneous Conditions.—Many other diseases have been studied in various clinics, but it is impossible to discuss them at length. Grafe [78] has found an increase in metabolism in cancer and only a moderate increase in low-grade fevers. Various diseases of the pituitary have been studied by Falta [64] in Vienna, by Means [24] in Boston, and by the Sage staff in Bellevue Hospital.[79] Results are variable and no conclusions can be drawn as to any marked change in the respiratory metabolism, except that in acromegaly there is a slight but fairly constant rise in heat production. The metabolism of women just before and just after childbirth was studied most carefully by Carpenter and Murlin [80] using one of the calorimeters in Benedict's laboratory. The effect of drugs on the heat production of men has been studied

by Loewy,[81] Linhard [82] and others and important work is now being done on this subject by Edsall [83] and Higgins and Means.[84]

We must not forget that much of our knowledge of the respiratory metabolism in disease is due to the work done on animals and normal men. We need only mention the researches in this country of Lusk, Benedict, Murlin, Carpenter and many others.

GENERAL CONSIDERATIONS

We have seen that the respiration calorimeter measures the heat production by the independent methods of direct and indirect calorimetry. The direct method must remain the standard in the long run and any significant and consistent divergence of the indirect method in any particular disease would indicate a marked change in the intermediary metabolism. It would show that the food-stuffs were not broken down to the same end-products with the same liberation of heat as in normal subjects. Such changes in disease have often been considered very seriously, particularly by investigators who, on account of defective technic, have obtained abnormally low quotients. The calorimeter has obtained no such quotients except in severe diabetes, where they are to be expected. The direct and indirect calorimetry have agreed very closely when we consider the technical difficulties in short observations with sick patients. Until a very few years ago no one would have dreamed of trying to make the two methods agree in periods shorter than six hours. At that time the calculations were not even carried out, but if we now go over some of the work in Benedict's laboratory [80] as early as 1911 we find excellent agreement in two-hour subdivisions of four-hour experiments. With the Cornell calorimeter built by Williams,[9] Lusk has been able to obtain remarkable agreement in periods as short as an hour with dogs and a dwarf. Howland [31] has obtained similar results with babies. When the totals obtained on all the normal controls studied in the Sage calorimeter are compared it is found that the two methods agree within 0.2 of 1 per cent. With the experiments two to three hours long, such as are used with patients, certain technical errors have a tendency to make the direct calorimetry too low, and we find it 1.1 per cent. lower

in typhoid fever, 1.8 per cent. in exophthalmic goitre, 2.3 per cent. in diabetes, 3.3 per cent. in anæmia, 1.9 per cent. in the group of cardiac and nephritis patients. These divergences are within the limits of technical error; they are so small that we can be certain that the law of conservation of energy holds good in disease and can also be sure that there are no profound and unsuspected changes in the intermediary metabolism. It is not possible to rule out small changes which might be concealed by the limits of technical error. The chief function of the calorimeter has been to show that the principles of indirect calorimetry are correct when applied to disease. Another function is to make it difficult for overenthusiastic theorists to promulgate wild hypotheses.

The results of work with calorimeters and other types of respiration apparatus must build the foundations of our theories of the nutrition of patients. If we desire to administer to any given patient his exact requirement it is necessary to make several experiments on the patient himself. It is very doubtful if the most experienced observer can guess the heat production of a sick man within 20 per cent. Still, it is possible to make rough calculations which will allow the physician to reckon out the caloric requirements of his patients somewhat more exactly than the trained nurse. We can use as a standard the basal requirements of healthy men between the ages of twenty and fifty and add 10 per cent. for the stimulation of food and about 10 per cent. for the usual activity of a patient confined to bed. Under these conditions the food requirements for the day would be 1800 calories for a man of 125 pounds, 2000 calories for 150 pounds, and 2200 calories for a man of 175 pounds. Bearing in mind the numerous factors already discussed we can add the various percentages according to the disease and its severity. If the patients are permitted to get out of bed, or if they are very restless, we must allow for an increase of anywhere between 20 and 100 per cent. during the hours when they are moving about.

Some observers have attempted to determine the total food requirement in disease by observing the intake and output of nitrogen. Such experiments in nitrogen equilibrium must be continued for weeks to give much evidence on this point. The

work at Bellevue Hospital in typhoid fever[38] has shown that a negative nitrogen balance may exist when the caloric needs are more than covered by the food.

In many cases the fluctuations of body weight serve as a guide to the nutritive condition of the body. We must not place too much reliance on short observations. Rapid changes in weight are due in great part to changes in the water content of the body. Benedict[2] quotes the case of a football player who lost 14 pounds in a game one hour and ten minutes long. Only one-quarter pound was due to the oxidation of solids, 13¾ pounds was water loss. We are familiar with the retention of large amounts of water in œdema and are familiar with the rôle of salts, but we do not realize the frequency of invisible œdema. A diet rich in carbohydrates causes the body to retain considerable amounts of water; a fat diet has the opposite effect.

Many clinicians are in the habit of measuring the fluid intake and urinary output under the impression that they are determining the water balance. Patients in the calorimeter eliminate water through skin and lungs at a rate which varies between ½ and 1 liter a day. The fluids of the diet form but a small part of the water intake. Rennet, a solid food, contains just as much water as milk. Potatoes are 75 per cent. water, tomatoes 94 per cent. An accurate water balance is one of the most difficult problems in experimental metabolism and we must not place too much reliance on changes in weight unless followed for long periods.

In reviewing the subject of metabolism in disease one can see that clinicians too long contented themselves with urinalyses and measurements of intake and output. Now they are thinking in terms of the blood and the fluids which bathe the cells. It is certainly worth while to go one step farther and study more closely the respiratory metabolism which tells of the results of the activities in the tissues themselves.

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THE METHOD OF GROWTH OF THE LYMPHATIC SYSTEM *

PROF. FLORENCE R. SABIN
Johns Hopkins University

IN SELECTING a title connected with the general subject of the lymphatic system, I have chosen to emphasize the phase of the subject with which the anatomist of to-day is concerned. As a matter of fact, in studying the problem of growth he is seeking to understand the nature of the lymphatic capillary. This is no new problem, but rather has dominated the study of the lymphatic system for nearly three hundred years. The colorless fluid of the tissues was called lymph long before lymphatics were discovered. It was thus natural that when vessels were found containing this fluid they were called lymphatics. As soon as the lacteals and then the general lymphatics were discovered, the question arose in regard to the nature of these new vessels, what was their extent and how they ended in relation to the surrounding tissues. At first the lymphatics were thought to begin in wide mouths in the walls of the various cavities of the body and then, as these openings proved difficult to find, attention became focused on the relation of the lymphatics to the tissues. The number of terms which have been used in seeking to analyze the relation of the lymphatics to the tissues, for example lymph-radicles, lymph-rootlets, lymph-spaces, parenchymal spaces, tissue-spaces, will serve to illustrate how persistent has been the quest of the anatomist to understand the lymphatic capillary. Stated in other terms, this is the time-honored question of open and closed lymphatics. In presenting to you the conception of lymphatic capillaries as definite vessels completely lined by endothelium, and related to tissue-spaces just as blood-capillaries are, it will be necessary to emphasize first the importance of tissue-

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spaces. Indeed the general subject of tissue-spaces as important systems in the body, related to blood-capillaries and to lymphatic capillaries in function, is, I believe, nowhere sufficiently emphasized in the literature.

It is well known that the plasma of the blood is constantly exuded from the blood-vessels into the tissue-spaces, so that all the cells of the supporting tissues, as well as the special cells of each organ, are bathed in fluid. Moreover, it is obvious that with all the varying activities of the cells of the body, the fluid becomes laden with different nutritive and with different stimulative substances and with different waste products, so that it varies widely in its composition. The subject of tissue-spaces—meaning not empty spaces but spaces which always contain fluid—is by no means simple. There are primarily the general, small spaces to which I have just referred, between all of the fibres and cells of the connective tissues and between the parenchyma of each organ and its supporting tissues: but there are also special systems of great spaces, which arise from the small spaces by a definite method, which have a definite structure and contain a fluid which is different from the other fluids in the body, such, for example, as the subarachnoid spaces which surround the central nervous system.

That the cerebrospinal fluid is secreted by a special organ and contains certain products of internal secretion is now known. The pia-arachnoid membrane has been shown by Weed [1] to have an extremely interesting structure and development. I will mention here only the very important arachnoidal villi, which are lace-like projections of the arachnoid into the dura. They lie along the dural veins and lead to the dural sinuses. These villi, which he has shown to be the organs of absorption for the cerebrospinal fluid, are covered with a layer of mesothelial cells, which become more abundant at the tips, forming cell nests.

Other great systems of spaces are found in the internal ear and in the eye. The scala tympani and scala vestibuli of the cochlea have been called perilymphatic spaces, though they have no relation to the lymphatic system. These spaces of the ear have just been shown by Streeter [2] to have a most interesting

development. The scala tympani and scala vestibuli are formed from spaces in the mesenchyme which at first become slightly larger than the usual spaces and then coalesce into still larger spaces. Moreover, this process is not indefinite but has two distinct places of origin, one between the sacule and the oval window and the other between the cochlea and the round window. From these two areas the formation of the two great spaces of the cochlea proceeds in a definite and constant direction, so that a model of their form from one specimen is the same as that from any other specimen of the same stage. Moreover, when studied in sections this process appears to be a gradual dilatation of preexisting tissue-spaces, with a disappearance of more and more of the original connective-tissue syncytium, rather than being caused by a differentiation of the mesenchyme cells forming the border of these spaces. As the cavity thus formed reaches its ultimate dimensions some of the remaining mesenchyme cells do differentiate to form a mesothelial lining. I emphasize this method of the formation of a cavity out of mesenchymal spaces for the reason that I believe it to be essentially different from the method of formation of blood-vessels.

Again in the eye there are two cavities having an entirely different development. Posterior to the lens is a space filled with fluid, which begins not by a hollowing out of tissue-spaces in mesenchyme, but as a definite differentiation of a primitive vitreous body by the retina. In the formation of this body the mesenchyme is only secondarily concerned. On the other hand, the history of the aqueous chamber of the eye is analogous to that of the formation of the cerebrospinal system of tissue-spaces.

Along the pathway of the blood-vessels of the central nervous system are special chains of tissue-spaces, lined by an indefinite mesothelium, but arranged in sufficiently definite lines to have received the name of perivascular lymphatic spaces. These spaces, however, have no relation to lymphatics and should be called perivascular tissue-spaces. Along the nerves also are chains of spaces which can be injected in the embryo, and which may be termed perineural spaces. Similar chains of connecting spaces have been injected by Lhamon[8] along the course of the Purkinje fibres of the

heart. Beside these very interesting special systems of tissue-spaces there is a group of great spaces which is still better known —namely, the great serous cavities of the body. These cavities, which form as a dilatation of spaces in the mesenchyme, have also a definite embryological history, a definite cellular wall of mesothelium, and a special very scanty content of fluid.

In order to analyze the relation of the general tissue-spaces and of these special systems of large tissue-spaces which develop out of the general ones, it is necessary to submit them all to some type of experiment. Fluids containing a suspension of minute granules, or true solutions whose location can be detected subsequently by the precipitation of granules injected into these various spaces, give widely and astonishingly different results. Weed has carried out a very interesting series of experiments of injections into the subdural and subarachnoid spaces. In these experiments he injected a solution of potassium ferrocyanide and iron ammonium citrate, at the same time withdrawing an equivalent amount of cerebrospinal fluid, to eliminate phenomena due to pressure. He found that when the granules of Prussian blue were precipitated by an acid fixing agent, they were in the meshes of the arachnoidal villi, within the cells of the nests of mesothelium at their tips and within the dural sinuses. On the other hand, when he produced a cerebral anæmia by bleeding, the fluid was sucked into the special and very important tissue-spaces that surround the nerve-cells. These experiments demonstrate conclusively that the central nervous system has a special system of tissue-spaces beginning, one might say, with the spaces surrounding every individual nerve-cell of the brain, extending into the subarachnoid area and draining not by lymphatics but by another special system of absorbents, namely, the arachnoidal villi, into the cerebral sinuses. Wegefarth [4] has shown that the anterior chamber of the eye has a similar system of absorbents, the pectinate villi. These lead to the canal of Schlemm, a vein analogous to the cerebral sinuses.

When injections are made into the peritoneal cavity the results vary widely, according to the nature of the fluid injected. As a matter of fact our knowledge of this important subject is far

from complete, but it has been shown that certain true solutions are absorbed by the blood-vessels. On the other hand, it is known that granules are in large part taken up by special, large, phagocytic cells, some of which pass into the lymphatics of the diaphragm. This gives a suggestion of a possible differentiation in absorption between blood-vessels and lymphatics. Indeed, a partial differentiation in function is a most familiar phenomenon: I refer to the villi of the intestine, where almost all of the fat passes into the central lacteal while the carbohydrates pass directly into the blood-stream. It is well known, on the other hand, that when a needle is introduced into certain areas under the skin or into specific layers of many of the organs and a fluid containing granules is injected, the granules always appear in the lymphatic trunks which drain the area. What is the difference between tissue-spaces which are drained by lymphatics and those which are not? What is the difference between areas in which injections always show lymphatics and those which never show lymphatics? What is the nature of the fluids which pass through the lymphatics and those which do not? In other words, exactly what happens at the point of the needle when an artificial œdema is produced? This I understand to be the meaning of the main problem connected with the lymphatic system—the solution of the enigma of the mechanism of absorption. The difficulty of the problem was well expressed by Bartels [5] as late as 1909, when he said that the relation of the lymphatic capillary to the tissue-spaces was a philosophical rather than an anatomical problem. My understanding of the recent work on the lymphatic system is that it tends to take the system out of the realm of the mythical and to make it a definite anatomical entity. The investigations of the last fifteen years have demonstrated that the blood-vessels are the primary absorbents, and that subsequently partial systems of absorbents develop, such as the arachnoidal villi and the lymphatics which drain into the veins.

I have been greatly interested in the attempts of the earliest anatomists to solve the problem of absorption. They brought to the subject of tissue-spaces and the fluid within them, a great freshness of interest and constantly sought to understand the

meaning of their various observations. They saw the arteries become smaller and smaller, they were familiar with lymphatic trunks and with some lymphatic capillaries. What then was more natural than to assume that when the arterioles became so small that the corpuscles could not enter, there were still smaller vessels which carried the plasma over into the lymphatics? These tiny hypothetical vessels were called "vasa serosa." A belief in their existence was held throughout the 18th century, and was not overthrown until the discovery of cells by Schwann in 1839. Schwann believed that the mesenchymal cells were hollow, and from this idea, Virchow formulated the theory that hollow connective-tissue cells spanned the gap between the blood-vessels and the lymphatics. Then followed the discovery by von Recklinghausen that the wall of the lymphatic capillary is composed of cells. Von Recklinghausen thought that silver impregnations showed that lymphatics spread out as lymph radicles or lymph rootlets into the tissue-spaces. At first His believed in these lymph radicles, that is, in open lymphatics, but von Recklinghausen's discovery of endothelium led him to a conception of a lymphatic capillary as a definite, closed vessel, this conception being confirmed by his own experience with injections. If lymphatics open out into tissue-spaces, every injection of a capillary plexus with a non-diffusible fluid should spread out into tissue-spaces and obscure the vessel—which is most obviously not the case. Thus von Recklinghausen's discovery served to bring up anew the question of open and closed lymphatics.

During the present century it has become evident that some light might be thrown on the obscure question of the relation of tissue-spaces to lymphatic capillaries through the study of their development. The first general hypothesis concerning the origin of the lymphatic system in the embryo was that fluid exuded from the peripheral blood-vessels and gradually hollowed out channels. As the fluid increased, these vague channels were thought to extend from the periphery to the centre and then to establish connections with certain veins. This hypothesis was made concrete by Gulland,[6] who found large empty vessels in the skin of embryos about 4 cm. in length, and thought them to be

the first lymphatics. In reality the lymphatics begin much earlier. This general hypothesis was to some extent modified by studies of Budge [7] and Sala.[8] Budge injected the extra-embryonal cœlom in early chick embryos, and got patterns of injection in the area vasculosa vaguely simulating lymphatics. These patterns we now know were produced by fluid passing out of the cœlom into the net-work of spaces between the plexus of blood-capillaries. Budge then made beautiful injections of true lymphatics in much later stages, and to explain his observations built up the hypothesis that there is a primitive lymphatic system associated with the body cavity and a later, secondary system of definite ducts. The thoracic duct he believed formed the connection between these two systems. These observations of Budge, which we now know to be incorrect, are, however, of great interest to the embryologist—representing as they do the earliest groping in darkness in hope of finding the first lymphatics. The work deserves emphasis also as the only basis of all the erroneous theories surrounding the idea that the body cavity is in some especial way a part of the lymphatic system.

Another very interesting attempt to find the first lymphatics is shown in the work of Sala, who studied the origin of the posterior lymph-hearts in the chick. We know now that these lymph-hearts arise as endothelial buds from the walls of the coccygeal veins and that these buds develop into a plexus, which becomes a pulsating lymph-heart. Sala, working with this rapidly developing plexus, somewhat vaguely appreciated its relation to the veins: he described a hollowing out of cavities in the mesenchyme near the veins and then said that in the last analysis these cavities in the mesenchyme were from their first appearance nothing but terminal dilatations of the veins. However, he concluded that the lymphatics begin as excavations in the mesenchyme which soon join the veins. The confusion in Sala's description is now easily understood. Dominated by the theory that lymphatics were tissue-spaces he could not analyze the evidence that they were from the start connected with the veins, and so described them as both veins and tissue-spaces. He made it clear, however, that he believed that the ducts were formed from chains of tissue-

spaces hollowed out in the mesenchyme and lined by flattened out mesenchyme cells. Sala's work, however, places the first lymphatics close to the veins.

Sala's work was published in 1900, and during that year I was working on the development of the lymphatic system.[9] I began the investigation by injecting the foot-pads of young pig embryos. This procedure never fails to demonstrate lymphatics in the adult, and the same is true of fetal stages, but it was soon found that in embryos less than 3 cm. in length it was necessary to introduce the needle nearer the central veins in order to find lymphatics. By a long series of such injections the fact was gradually established that the skin of the embryo is invaded by lymphatics from two general regions—the neck and the groin. By noting the lines of growth of these invading vessels it was possible to obtain injections, showing the extent of the invasion of the skin for each stage. Moreover, in making these injections into the translucent skin of the embryo it became evident that in order to fill the lymphatics the needle must be introduced at a very exact level. When the needle cuts the lymphatics, the vessels can be seen to fill up from the oblique opening of the needle, without any extravasation if the pressure is light. If the needle is entered too superficially a bleb is always formed: if too deeply, the injection mass spreads out in straight lines, very characteristic and very different from lymphatics. These observations emphasize the lymphatic capillary as a definite vessel located at a specific level. Through a long series of such injections these definite lymphatic vessels were traced back to tiny buds close to the veins. The theory was then advanced that the entire lymphatic system consists of definite vessels of endothelium, which grow as blind buds from the endothelium of the veins and partially invade the body. The theory throws the emphasis on endothelium as the essential tissue of the lymphatic system, and premises that the endothelium of the lymphatic system is derived from the endothelium of the veins. This means that lymphatic vessels arise as an active growth of endothelial cells and are not formed by a passive dilatation of spaces. The outgrowth theory has not been established without opposition. There has been,

indeed, a vigorous effort in this country to re-establish the older hypothesis of the origin of lymphatics from tissue-spaces, but in my judgment these efforts have not been successful.

I shall now outline briefly certain facts which have been established concerning the development of the lymphatic system. The lymphatic system begins in the human embryo of about 10 mm. in length, that is, during the sixth week of development. The first lymphatics are blunt buds which come from the internal jugular veins at the root of the neck. They are filled with blood which backs into them from the vein. These buds soon establish connections with each other and form a plexus which develops into a large sac, having its base on the internal jugular vein and arching into the posterior triangle of the neck. From this sac, which is astonishingly large, lymphatics grow out to the skin of the head and neck, to the thorax and arm and partially invade the deep structures of the head. From the portion of the sac in the posterior triangle of the neck, vessels grow forward and form an extensive plexus along the external jugular vein. The knowledge of the form of this sac, of its position with reference to the internal jugular vein, and the pattern of the plexuses of lymphatics which develop from it, has unravelled the complicated and puzzling relations of lymphatic ducts to the chains of lymph glands in the neck. The sac itself is transformed into different groups of lymph glands which might be analyzed as the primary lymph glands of the neck, and these primary lymph glands bear a definite relation to the secondary glands which form along the ducts growing out from the sac.

At a slightly later stage—in embryos of the seventh week, approximately 20 mm. in length—a series of lymphatic buds develop from some of the abdominal veins. These early buds have proved more difficult to study than the jugular buds,—first, because the veins from which they arise are more complex and were less well known, and, secondly, because their deep position has made direct observation in the living embryo and direct, precise injections practically impossible. Therefore our knowledge of the extent and origin of the abdominal lymphatics from different veins is still far from complete. Certain very interest-

ing observations by Silvester [10] on monkeys and by Job [11] on rats, show that in these forms certain lymphatic ducts drain permanently into the inferior vena cava, the iliac, the renal or the portal veins suggesting a multiple origin of lymphatics from abdominal veins. The main abdominal lymphatics, however, begin as a retroperitoneal sac which develops from a vein connecting the two Wolffian bodies. This vein ultimately forms a part of the inferior vena cava. This large retroperitoneal sac furnishes the key for the study of the abdominal lymphatics. The lymphatics of the skin of the abdomen and for the legs grow from paired iliac sacs. The retroperitoneal sac and the iliac sacs become connected with the left jugular sac by means of the thoracic duct, which grows from the left jugular sac and from the abdominal lymphatics, and is complete in embryos about 25 mm. long. There is thus formed a primary lymphatic system of sacs connected by the thoracic duct; this system in most mammals drains into the internal jugular veins on either side. From the primary sacs, a plexus of capillaries invades the body. In a general way, the vessels from the jugular sacs grow to the head, thorax and thoracic viscera; those from the retroperitoneal sac to the abdominal viscera, and in part to the thoracic viscera; and those from the iliac sacs to the abdominal walls and legs.

The injection of these invading plexuses of lymphatics from the sacs outward is possible in the embryo, though it is impossible in the adult, owing to the fact that the early vessels are without valves. In a general way it may be stated that by the time a fœtus has reached the length of 5 cm., almost the entire skin has been invaded by a single plexus of lymphatic capillaries and the organs have received their primary lymphatic vessels. At this stage of embryonic development, injections of any part of the lymphatic plexus spread out in all directions, so that theoretically the injection of any capillary might fill the entire system. I have injected the thoracic duct, for example, from the skin of the thorax, the injection mass passing around through the iliac lymphatics; or again I have injected the lymphatics of the skin by puncturing the thoracic duct. This complete anastomosis of the primary, lymphatic capillary plexus of both the superficial

and the deep systems in the embryo seems to me to be of considerable importance.

To illustrate the development of the lymphatic system to an organ and within an organ, I shall describe Cunningham's [12] work on the lymphatics of the lung. He has found that lymphatics approach the lung from three sources: from the two jugular sacs there are right and left lymphatic trunks, and from the retroperitoneal sac there are vessels which come up behind the diaphragm. The ducts which grow down from the neck meet in a plexus which surrounds the trachea. In the primitive lung, the general pattern of the organ is simple; it is obviously blocked off into large lobules by wide connective-tissue septa. In the centre of each lobule are the bronchus and the artery, in the septa are the veins. At the hilum the tracheal lymphatics divide into three plexuses, one spreading onto the pleura, a second following the bronchi and arteries, and the third the veins. The plexus which follows the veins grows rapidly to the pleura and spreads around the border of each primitive lobule blocking off the pleura into polygonal areas. From this pattern the pleural lymphatics develop. The pleura is blocked off into its polygonal areas by the lymphatics when the embryo is about 5 cm. in length. At a much later stage, when the bronchi begin to develop atria and air sacs at their tips, the lymphatics grow down the centre of the lobule along the bronchi. Just where the atria begin, the lymphatics turn sharply from the bronchi and pass out to the septa, so that the walls of the air sacs are without lymphatics.

The lymphatics of the diaphragmatic surface of the pleura grow up behind the diaphragm from the retroperitoneal sac, and injections of this surface of the lung in later stages fill up the pre-aortic, abdominal lymph glands. This relation of the pleural lymphatics to the abdominal lymphatics I believe to be of importance.

The development of the ducts to the intestines, and their differentiation within the intestinal wall into the ultimate lacteals of the villi, have also been worked out. The method of injection in the embryo affords an excellent opportunity to test the present belief in the partial invasion of organs by lymphatic

vessels. For example, lymphatics have not been demonstrated in the adult liver beyond the capsule and the connective-tissue septa, nor in the spleen beyond the capsule. It is well known that lymphatics are abundant in tendons; but they have not been demonstrated in striated muscle. On the other hand, it has been definitely shown, both in the embryo and in the adult, that there are no lymphatics in the central nervous system.

To this very general account of the lymphatic system in the mammal, certain interesting facts from comparative anatomy must be added. It has long been known that there are pulsating lymph-hearts in the amphibia. These lymph-hearts arise as lymph sacs from the vertebral veins in the neck and from the coccygeal veins at the root of the tail. These sacs are close to the myotomes and develop striated muscle in their walls. In the birds there is a very interesting lymphatic system. There is a jugular lymphatic plexus which later becomes a lymphatic-gland, and a caudal pulsating lymph-heart, which develops from the coccygeal veins. In mammals the lymph sacs develop into groups of lymph-glands, which may be called the primary glands for each region, while secondary glands develop along the lymphatic ducts.

In this brief résumé of the lymphatic system I have given only facts which can be clearly demonstrated. There are these sacs lying next to the veins, and if injections are made from them one can demonstrate a gradually increasing plexus of vessels. These facts, however, but lead us on to seek their meaning. What are lymphatic capillaries, how do they arise, and how do they grow? There is general agreement that the lymphatics arise from certain centres and grow toward the periphery; but there are two theories as to how they grow. The theory which I hold is that the lymphatics arise from the endothelium of the veins and grow by the multiplication of endothelial cells. The opposing theory holds that the lymphatics arise from tissue-spaces and grow by adding on new tissue-spaces; that beyond the tip of a definite completed vessel, which can be injected, are tissue-spaces which will be added to the capillary.

It is here necessary to submit the different types of method and the nature of the evidence which has been brought forth

under the stimulus of these two theories. Some of the methods are direct, some indirect, but in all there is an effort to understand the nature of that very interesting and important tissue, the endothelial cell.

First, in regard to the nature of the earliest lymphatic buds, it is clear from sections, both of mammals and of birds, that these buds are lined by endothelium, but it proved very difficult to determine from sections that these buds were from the beginning connected with the veins. Eleanor Clark,[13] however, was able to test this point in the case of the lymphatics of the chick by developing a method for observing the tiny red buds in the living embryo. Into these lymphatic buds she injected a few granules of ink, and then observed the granules entering the vein. Moreover, in the amphibia Fedorowicz [14] has traced each step of the origin of the first lymphatic buds from the veins, by specific differences between the endothelium and the mesenchyme.

From these early lymphatic buds it is possible to inject an increasing plexus of lymphatic capillaries as the embryo develops, and by this method to follow the lymphatic capillaries to their form in the adult, in the few places where that form is known. On this evidence was based the theory of the centrifugal invasion of the body by lymphatics.

The next method of study which occupied the attention of the group of anatomists who were trying to follow the development of the lymphatic system was a comparison of the adequacy of the method of injection with the adequacy of the method of reconstruction of lymphatics from serial sections, as applied to the problem of growth. This long series of studies followed an observation of Lewis [15] that if the lymphatics were reconstructed from sections they would appear as isolated vesicles for which no connections could be found. This is the experience of all who attempt to reconstruct an uninjected capillary plexus from sections, and therefore it has been necessary to test the limitations of the method. It is claimed that the method of reconstruction reveals more lymphatics than can be shown by the method of injection, as it shows not only all the lymphatics which can be injected but also the spaces that will be added to the plexus later.

Moreover, it is on the evidence of reconstructions that the theory of the growth of lymphatics by the addition of tissue-spaces is based. It is true, of course, that injections would not fill up solid sprouts of endothelium, and everyone who has made injections of lymphatics is familiar with the difficulties of obtaining perfect specimens, but it has been demonstrated that when an area is chosen which can be adequately injected, more of a capillary plexus can be shown than can be reconstructed. For example, Eleanor Clark [16] has published a picture of an injection of the jugular lymphatic plexus of a chick which showed a far more extensive plexus than was demonstrated in a reconstruction of the same stage, previously recorded by Miller.[17] The two pictures, side by side, afford a striking contrast. The amount of the plexus which can be demonstrated by reconstruction increases very much if an oil immersion lens is used, but the method, though one of the most important aids in embryology, is entirely inadequate to test the method of growth of capillaries. No one would regard it as adequate to determine an entire plexus of blood-capillaries even where their pattern is well known.

It is, I think, obvious that the only adequate method for the study of the growth of capillaries is to observe them in a living specimen; and in this connection we have a long series of valuable observations on the classical object, the living tadpole's tail. Capillaries were first seen in the tadpole's tail by Schwann, and were first differentiated into two types, blood-capillaries and lymphatic capillaries, by Kölliker. During a long series of studies with this object, by Remak, Sigmund Meyer and others, and finally by Eliot R. Clark,[18] with greatly improved methods, two facts have become established: first, that endothelium is contractile and, second, that the vessels grow by the cell-division of their own walls. Clark was able to watch a given lymphatic for several days and to observe that the wall put forth tiny processes of protoplasm, which we term sprouts, that the nuclei of the cells divided and wandered into the new sprouts, which developed into new vessels. He was able to plot out every mesenchymal cell in the neighborhood and to show that the growing sprouts of endothelium avoided rather than approached the proc-

esses of mesenchyme, and never incorporated them into their walls. Thus in the one place where natural conditions are such that every cell, or rather every nuclear area of a growing vessel, and every mesenchymal cell can be identified, it is without question true that both blood-capillaries and lymphatic capillaries grow through the proliferation of their own walls.

The method of growth of capillaries may thus be regarded as established. But this is not the whole problem for the embryologist. Under development he must consider not only growth but also the original differentiation of tissues. In embryology it has become clear that there is a gradual differentiation of tissues from a common cell mass, and that after a tissue is once differentiated it increases by cell-division. This conception of differentiation was clearly stated by von Baer in 1828. He called the process histological differentiation. Thus development consists in the differentiation of tissues followed by growth. The most recent work on the lymphatic system demonstrates that the period of differentiation of endothelium is the period of the origin of the blood-vessels, and that this period has long since passed when lymphatics begin. Lymphatics do not differentiate from mesenchyme but grow from veins.

It is well known that methods have long been sought by histologists to distinguish endothelium from mesenchyme. If we could always distinguish endothelium in sections the problem would be practically solved, but the difficulty of determining lymphatic endothelium in the sinuses of lymph-glands, or vascular endothelium in the spleen-pulp, are too well known to need emphasis. These very difficulties lead us to the question, is endothelium differentiated from mesenchyme?

Efforts to distinguish endothelium from mesenchyme have not been entirely without results. For example, Clark has found that in the chick the nuclei of lymphatic endothelium can be distinguished from the nuclei of the mesenchyme by characteristic nucleoli. Again Kampmeier [19] has shown that both the venous and the lymphatic endothelium in the toad can be distinguished from mesenchyme at a certain stage by the presence of a greater number of yolk globules. Indeed, this differentiation of vas-

cular and lymphatic endothelium from mesenchyme was so striking as to convince Kampmeier that the lymphatics arose from the veins, though he had previously held the view that they arose from tissue-spaces.

These observations, valuable as they are, are not sufficiently universal to determine the nature of endothelium. The lymphatic endothelium grows from the endothelium of the veins; but since it varies slightly from the venous endothelium we may say that it is secondarily differentiated from it. This idea leads us directly to the most fundamental problem connected with the entire vascular system, namely, how does endothelium arise, and how do the first endothelial cells differentiate? The question of the origin and the growth of the lymphatic system will not be completely solved until its essential tissue endothelium is completely understood. This leads us to seek for the origin of the first blood-vessels.

The question of the origin of the heart and blood-vessels has a vast literature. Since the time of Wolff and Pander, it has been known that blood-islands in the chick arise in the wall of the yolk sac. Then His [20] discovered that blood-vessels arise by a differentiation of vasoformative cells or angioblasts. This is the fundamental point which recent work confirms. His having proved that angioblasts differentiated in the wall of the yolk-sac, and having seen that they did invade the embryo, advanced the hypothesis that all the angioblasts are differentiated in the yolk-sac and then invade the body from the embryonic membranes. The theory regarding angioblasts thus became centred around the idea of this invasion, and the more fundamental point was obscured. In recent years this theory that all of the vessels of the embryo are derived from the vessels of the membranes has been disproved by certain experiments of Hahn.[21] Hahn selected chicks in the stage of the primitive streak and burned out the membranes opposite the posterior end of the streak. In a few specimens which lived he found a small aorta and cardinal veins on the injured side of the embryo. These results have been confirmed by Miller and McWhorter [22] and by Reagan [23] on the chick and again by studies on the fish embryo by Stockard.[24]

It may thus be regarded as proved that blood-vessels arise both within the embryo and in the embryonic membranes.

Stockard then went on to attack the more fundamental problem, how does endothelium first arise? In studies made on the yolk-sac of the living fish embryo, he found that endothelium arises as spindle cells which differentiate out of mesenchyme. Moreover, he found that the endothelial cell was distinct in its origin from the blood-cell. This confirmation of the angioblast of His I regard as a very important contribution. It is clear, in following the work of His, that he made studies on the living blastoderm of the chick, but so far as I am aware McWhorter and Whipple [25] were the first to study the living blastoderm in a hanging drop preparation.

During the present fall I have been using this method. I find, just as did His, that blood-vessels begin by a differentiation of cells. It is difficult to be sure of the first cells in the living chick which become angioblasts, but by the time the first cleft appears which indicates the position of the two upper myotomes, there is an extensive plexus of bands of cells in the area vasculosa. In watching these bands of cells in the living specimen, I thought for some time that they could be differentiated by a slightly greater refractility than the rest of the tissue; but this did not prove to be an adequate criterion, for when the syncytium of mesenchyme forms in the later stages, it makes a network of tissue which is just as refractile. Moreover, in the study of the early vessels in the living blastoderm it is extremely difficult to tell which is the vessel and which the interspace. However, I found that the bands of endothelium, or the definite vessels which form from them, would suddenly change their appearance over wide areas, becoming intensely refractile and very granular and opaque. In this stage, which is so striking that it can be seen under low powers of the microscope, the vessels lose all appearance of being hollow; and I soon found that this was because every cell was entering into the phase of cell-division. This was proved by the rows of spindles in stained specimens.

The extent of cell-division in these chick embryos is most interesting. At times wide areas of the endoderm cells divide and

become so opaque as to entirely obscure the cells beneath, and one has to wait until the endoderm becomes clear again. The difference in the reaction of the bands of endothelium and the syncytium of mesenchyme to cell-division is a guide for the study of the early differentiation of blood-vessels. When the bands of endothelial cells divide the cells remain together; the outline of each cell becomes distinct, but they do not separate. In the case of the division of the cells of a syncytium of mesenchyme, however, many of the processes are withdrawn and the cell-body rounds up, so that it stands out as if it were an isolated cell, just as has been described by Margaret Reed Lewis in tissue cultures. Thus in areas in which it becomes very difficult to trace the ultimate strands of endothelium it may be necessary to wait for the phase of cell-division in one or the other tissue in order to make the distinction. In watching the vessels of the area vasculosa, one gets the suggestion that there may be a rhythm in cell-division. For example, if the area pellucida around the posterior end of the embryo be considered as divided into an inner and an outer zone, either all the vessels of the inner zone or all those of the outer zone may be found in cell-division at the same time.

The vessels of the original plexus increase in size by cell-division and new vessels are constantly formed within the plexus by numerous sprouts that grow out to connect its meshes. Beside this growth within the plexus, there is an active differentiation of new endothelial cells, which can be watched in the living chick. In the early stages, up to five or six somites, there is no syncytium of mesenchyme and the wandering cells are scanty in number. Individual spindle-cells are thus clearly seen. They divide and at once show the essential characteristic of endothelium—that is, the tendency to form bands. Either an individual cell, or bands of two or three cells, send out tiny processes toward the older bands of endothelium, which at once respond by sending out tiny processes to meet the new ones. Thus endothelium consists of cells which differentiate as spindle-cells from the mesenchyme, and show at once two characteristics, first a tendency to remain together after cell-division forming strands, and, secondly, a

tendency to join other similar bands of cells by protoplasmic processes. These bands of cells become blood-vessels.

It is, I think, clear that the question now to be solved is how long does endothelium continue to differentiate out of mesenchyme. It can be seen to differentiate in the living chick in all the stages I have yet studied, that is in the stages before the circulation is established. This covers approximately the first two days of incubation. As is well known, there is a group of anatomists—Maximow, Reichert and Mollier, and a group of American workers, notably Huntington and McClure—who believe that endothelium continues to differentiate out of mesenchyme possibly throughout life. From the evidence which I have previously given I think it much more likely that endothelium will prove to have a limited period of differentiation, followed by growth. The study of the origin of blood-vessels seems to me to emphasize again the endothelial cell and to show that the vascular system arises from a differentiation and growth of endothelial cells rather than by a dilatation of spaces.

In looking back over the history of the development of our knowledge of the lymphatic system, it is very clear that there have been periods of great activity followed by periods of rest. We are at present in a period of activity, and I should like to sum up what seem to me to be the results of the work of the last fifteen years. It has been shown that the problem of the origin of the lymphatic system is but a part of the general problem of origin of the vascular system. Lymphatics are modified veins, in the sense that they grow from the veins. The veins or the blood-capillaries are the primary absorbents and continue to take part in absorption throughout life. Up to the stage of six weeks for the human embryo, they are the only absorbents. Subsequently other systems develop, such as the arachnoidal villi and the lymphatic vessels, to assist in the function of absorption. The lymphatics only partially invade the body, and present indications point to the fact that their functions in absorption may be to some extent specific.

In an injection into the tissues of a dead organism it is essential to puncture the vessels of lymphatic plexus in order to fill the

lymphatics with a non-diffusible fluid. These injections demonstrate a complete wall, in the anatomical sense, which is ruptured only by increased pressure. In the living animal both true solutions and granules pass into lymphatic capillaries through the activities of endothelial cells or by means of wandering phagocytic cells.

This conception of the lymphatic system is at variance with the older idea of hazy lymphatic capillaries that faded off indefinitely through hypothetical lymph-radicals into the tissue-spaces. With the newer conception of definite lymphatic capillaries of endothelium it would be much better if we should revise the terms which developed in the period when our theories were vague and indefinite. In the first place, there are definite blood-capillaries, definite lymphatic capillaries and tissue-spaces. If we should reserve the name "plasma" for the fluid within the blood-vessels, "lymph" for the fluid within the lymphatics, and "tissue-fluid" for the fluids of the tissue-spaces, our conceptions would be greatly clarified. The term "tissue-fluid," meaning the fluid which is within the tissue-spaces in the living animal, should not be confused with the term "tissue-juice," by which the physiologist means the fluid which can be pressed out of the tissues. The tissue fluids are the most varied of all the fluids of the body, and include such special fluids as the cerebrospinal fluid, the aqueous humor, and the fluid of the serous cavities, as well as the general fluid of the less specialized tissue-spaces. It would also be a great gain in clearness if the term "endothelium" were restricted to the cells lining the entire vascular system, including the lymphatics, and Minot's term "mesothelium" was used for cells which line cavities which develop out of tissue-spaces.

The study of the lymphatic system throws emphasis on the importance of tissue-spaces. I am convinced that the understanding of lymphatic capillaries as definite structures, definitely located in restricted areas, forms a secure basis from which the varied problems of absorption may be solved.

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THE PRESENT SIGNIFICANCE OF THE AMINO-ACIDS IN PHYSIOLOGY AND PATHOLOGY *

DR. DONALD D. VAN SLYKE
Rockefeller Institute

CHEMICAL NATURE OF THE AMINO-ACIDS

THIS discussion is inserted because it will be necessary, for the ready understanding of the later parts, that speaker and audience shall have in mind from the same viewpoint two or three significant chemical characters of the amino-acids as a class.

We know, chiefly as the result of the researches of Fischer,[1] Kossel, and their collaborators, that the amino-acids are the units or building stones out of which the protein molecule is constructed. They are the final products obtained when proteins are hydrolyzed by strong acids, or by the action of pepsin, trypsin, and erepsin in the alimentary canal. In the characteristic points of their structure, the amino-acids are all alike. That is, they belong to a type, and we have only to understand the type in order to become fairly well acquainted with them all. We have placed on chart I what may be designated as a decapitated amino-acid. It is the portion of the molecule which is common to all the amino-acids, and its formula expresses the chemical properties which are characteristic of them as a class. Of these properties, the most striking are due to the occurrence in the same molecule of an amino group, with a basicity like that of ammonia, and an acid group with an acidity like that of acetic acid. Hence, from these two groups the name, amino-acid. The amino and acid groups are joined by a single carbon atom which serves as a bridge between them. This structure occurs in every amino-acid. The central carbon atom is the centre of the entire molecule. It is flanked on one side by the amino group, on the other by the acid group, a third valence is occupied by an insignificant hydrogen atom, while to the fourth, which in the decapitated formula is left pointing upwards and unattached, is fastened what we may term

* Delivered January 15, 1916.

the head of the molecule. This is different in every amino-acid. It is the source of the individuality of each. There are eighteen varieties of such heads, as may be seen by glancing at Chart 3, and, corresponding to them, eighteen distinct amino-acids, each possessing the common group characteristics indicated by the body, and, in addition, another set of chemical characters entirely belonging to itself, and indicated by the structure of the head.

CHART I

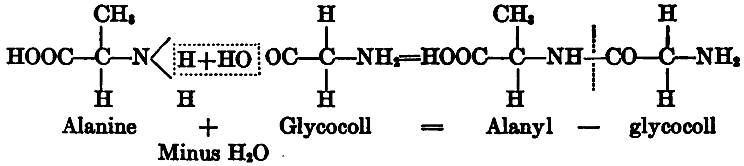
$$\overbrace{\underbrace{NH_2}_{\substack{\text{Amino}\\ \text{group}}}-\underset{\displaystyle H}{\overset{|}{\underset{|}{C}}}-\underbrace{COOH}_{\substack{\text{Acid}\\ \text{group}}}}^{\substack{\text{Place of attachment for}\\ \text{group by which each}\\ \text{amino-acid } \textit{differs} \text{ from}\\ \text{others}}}$$

Formula of a decapitated amino-acid.

Our interest centres mainly, however, on the family body and its characteristics. Of these we have already mentioned the basic and acid properties combined in the single substance. I would call your attention to one other, also dependent upon the simultaneous presence of amino and carboxyl acid groups, and this is the ability of the amino-acids to dovetail themselves together and form molecular chains of infinite length. It is this ability which makes possible the existence of such complex substances as proteins and protoplasm. We have indicated in Chart 2 the mechanism by which the linking of the units in the chain is accomplished. It represents the coupling of two of the amino-acids, alanine and glycocoll. We see that the amino group of the alanine condenses with the acid group of the glycocoll, with elimination of a molecule of water. The result is that the two amino-acids are linked together and form a peptide, alanylglycocoll, called a dipeptide because it contains two amino-acids. This peptide, however, like the original amino-acid, still contains one free amino group at one end and an acid group at the other. It can, therefore, couple on another amino-acid at either end. These could still condense with two more, and so on *ad infinitum.*

Protein molecules are chains composed of scores or hundreds of amino-acids joined together in this way.

Chart II



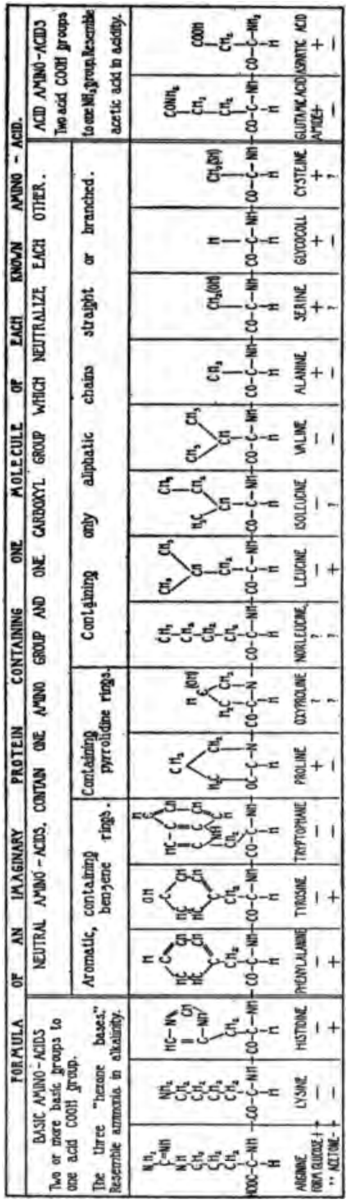
Coupling of the amino-acids, alanine and glycocoll, to form the di-peptide, alanyl-glycocoll.

When proteins are hydrolyzed or digested, by trypsin for example, the links of the chain are broken apart and we have first somewhat shorter chains, the albumoses, then still shorter chains, the peptones, which are mixtures of peptides, and finally the separate amino-acids. This successive breaking down of the long protein chain into shorter chains and finally into the separate links constitutes the process of digestion. The building up of new protein consists of the reverse, namely, the linking together of the amino-acids into new chains.

To show at the same time the nature of the protein structure and of the different amino-acids which take part in it, I have placed on Chart 3 the structural formula of an imaginary protein containing one molecule of each of the eighteen known amino-acids. We see along the bottom of the row the repetition of the familiar family body, the central carbon atom flanked in each case by the accompanying amino and acid groups. Above, however, we have the individual heads of the different units in great variety. We might liken the protein chain to a long train of autos, all with small, black, uniform bodies, but with tops of eighteen different shapes, and of three different colors according to whether the properties they carry are acid, basic, or neutral. It will be noted that among the amino-acids the neutral party is in the great majority, which fact accounts in part for the approximately neutral reaction of most proteins.

Whenever a peptide linking in the protein chain is broken by hydrolysis we have at once one amino group and one acid group

CHART III


FORMULA OF AN IMAGINARY PROTEIN CONTAINING ONE MOLECULE OF EACH KNOWN AMINO - ACID.
BASIC AMINO-ACIDS
Two or more basic groups to one acid COOH group.
The three "hexone bases." Resemble ammonia in alkalinity.
NEUTRAL AMINO-ACIDS, CONTAIN ONE AMINO GROUP AND ONE CARBOXYL GROUP WHICH NEUTRALIZE EACH OTHER.
Aromatic, containing benzene rings.
Containing pyrrolidine rings.
Containing only aliphatic chains straight or branched.
ACID AMINO-ACIDS
Two acid COOH groups to one NH2 group. Resemble acetic acid in acidity.
ARGININE
FROM GLUCOSE
ACETONE
LYSINE
HISTIDINE
PHENYLALANINE
TYROSINE
TRYPTOPHANE
PROLINE
OXYPROLINE
NORLEUCINE
LEUCINE
ISOLEUCINE
VALINE
ALANINE
SERINE
GLYCOCOLL
CYSTEINE
GLUTAMIC ACID AMIDE
ASPARTIC ACID

set free. Chemically stated, the hydrolysis or digestion of a protein consists in the splitting of some or all of the peptide linkings between its amino-acids, with the formation of new acid and amino groups in exact proportion to the extent of the digestion. In order to determine the occurrence and extent of digestion, with exactness, therefore, we must determine either the amino groups or the acid carboxyl groups that are formed by the process. Only by such means can we obtain results capable of exact chemical interpretation. The various physical methods of colloid coagulation, viscosity determinations, precipitation, etc., useful though they have been, are only rough and indirect measures of the chemical process which constitutes digestion. For a direct measure we must determine either the amino or the acid groups which are set free. Furthermore, our only chemical means for estimating the complexity of any intermediate product, such as a peptone or albumose, lies in determining the ratio between the free amino or acid groups which it possesses and those which are found after it has been completely hydrolyzed. Thus the amino nitrogen of a dipeptide, composed of two amino-acids, is doubled by hydrolysis, that of a tripeptide is tripled, of a tetrapeptide quadrupled, etc.

All of the above facts concerning the relationship between the progress of digestion and the uncovering of amino and carboxyl groups were recognized over ten years ago as soon as Emil Fischer[1] had demonstrated the peptide nature of the protein molecule. As the result of this knowledge, the desirability for quantitative methods for the determination of either amino or carboxyl groups became evident. As generally occurs, when the need became clear the methods were invented. Since the use of these methods is most intimately connected with the experimental work of which I shall speak, I shall stop here for a moment to discuss the two which have found most general application.

The first was published by Soerensen in 1908.[2] It was based on the fact, discovered by Schiff,[3] that formaldehyde added to the water solution of an amino-acid combines with the amino group, and that in consequence the amino group loses its alkalinity. As the formaldehyde itself is neutral, the effect of the reaction is

to reduce the amount of titratable alkali, or increase the titratable acid, by an amount equivalent to the amino nitrogen present. Soerensen tested this method with practically all the known amino-acids, and worked out the details necessary for attainment of the most accurate results. In brief the formol titration of Soerensen is performed by rendering the solution of amino-acid neutral to litmus, adding formaldehyde, and then titrating against phenolphthalein the acid which has been set free by the removal of the alkaline capacity of the amino groups. The ingenuity and simplicity of this method led to its immediate adoption by biological chemists, and many investigations of value have already been conducted with it.

The second method was published by myself in 1909.[4] It rested on the well-known reaction of amines with nitrous acid, as the result of which the nitrogen of the amino group is transformed into nitrogen gas. In order to determine the amount of amino nitrogen present, therefore, one has merely to add nitrous acid and measure the volume of nitrogen gas which is set free by the reaction. The principle is similar to that of urea determination by the hypobromite method. We were able so to fix conditions that the reaction is complete in three minutes. A considerable amount of nitric oxide gas is evolved by spontaneous decomposition of the nitrous acid, and this gas is used to drive the air out of the apparatus before the amino-acid solution is admitted. At the end of the reaction the nitric oxide is absorbed by permanganate solution, and the pure nitrogen gas given off by the amino group is measured.

In the apparatus which finally proved most convenient the entire process can be carried out in a few minutes and results obtained with a high degree of accuracy. As compared with the formol titration, the nitrous acid method has the disadvantage that it requires a special apparatus. It has several advantages, however, in that the readings can be made with a higher degree of accuracy, that the determination is not interfered with by the presence of colored substances in the solution, and that accurate results can be obtained with extremely small amounts of material. With a micro-apparatus readings significant to .001 mg. of amino

nitrogen can be obtained, while a quarter of a milligram is as small an amount as can be determined by the formol method. Because of these advantages, which were important in the conditions under which we worked, we have used the nitrous acid method in our own investigations on the fate of protein digestion products in the body.

The table below gives an idea of the nature of the results obtained in following the course of a protein digestion. It will be noted that there is a slight amount of amino nitrogen present before any digestion has occurred. This is due to the fact that one of the two amino groups of the lysine is free in the protein molecule. This was demonstrated in our laboratory by Birchard, who showed that in a representative series of proteins an amount of free amino nitrogen equal in all cases to half the lysine nitrogen could be demonstrated by the nitrous acid method. It will be noted from Chart III that one amino group in the guanidine nucleus of arginine is also free. This guanidine NH_2, for an unexplainable reason, however, fails to give some of the characteristic reactions of amino groups in general. It does not react with nitrous acid, nor with formol in the Soerensen titration, and therefore is responsible for none of the free amino nitrogen that is noted in the protein even before digestion has begun.

TABLE I.—INCREASE OF AMINO N DURING TRYPTIC DIGESTION OF 4 PER CENT. EDESTIN SOLUTION

Hours	C.c. of N gas from 10 c.c. solution	Per cent. of Hydrolysis
0	1.20	0.0
2	7.62	14.8
4	8.92	18.2
20	12.52	27.4
80	19.56	47.3
Complete hydrolysis with HCl	40.25	100.0

The above finishes our discussion of the organic chemistry of the amino acids, and the methods used for their determination. We shall now turn our attention to a study of the fate of protein

digestion products in the body. This study has been guided by the conception of the relationship between proteins and amino-acids which I have just outlined, and was carried out to a large extent with the aid of the nitrous acid method for the experimental investigation of that relationship.

PHYSIOLOGY OF THE AMINO-ACIDS

Before entering upon an account of these researches I must acknowledge the debt to my collaborators, without whom a large part of the work would have been impossible. I refer to Dr. Gustave Meyer, who collaborated in all the work thus far published, and to Cullen and McLean, members of the Rockefeller Hospital staff, to whose efforts are due results that will be reported for the first time this evening.

It is furthermore a pleasure, as well as a duty, to acknowledge my indebtedness to Dr. Levene, for six years my chief at the Rockefeller Institute. The work detailed this evening is a direct outgrowth of Levene's own researches on the proteins, was carried out with the constant inspiration of his enthusiasm, the help of his counsel, and of his generosity in making available every facility which the laboratory afforded, even at times to the delay of his own immediate work, the ultimate sacrifice that can be taken from a spirit such as his.

At the time these investigations were begun the old Liebig theory of protein metabolism had already long been abandoned, and in place of it there was considerable confusion. Liebig's belief was very simple. He thought that the food proteins were incorporated directly into the tissues of the animal. The only necessary preparation was that of putting the proteins into solution in order that they might be absorbed, and this purely physical change was the sole object of digestion. The better understanding of gastric digestion, Kühne's discovery of trypsin,[6] and finally Cohnheim's[7] demonstration of the action of erepsin in reducing proteoses to amino-acids, led inevitably to the conclusion that food proteins undergo not only physical, but chemical change in the alimentary canal, namely, that digestion is a

hydrolysis, and that the hydrolysis proceeds partially, if not entirely, to the stage of amino-acids before the products are absorbed.

The results of a century of laborious research by many keen investigators from Spallanzani and Beaumont to Cohnheim may be summarized as follows: The proteins enter the stomach and are digested to the stage of albumoses; that is, the long protein chain of amino-acids is broken into somewhat shorter, but still very long, chains, and thereby the protein, which is usually insoluble, is transformed into soluble albumoses. The latter are not absorbed, however. London, working in Petrograd, has shown conclusively that no absorption takes place from the stomach during normal digestion.[8] The albumoses all pass down into the intestine, where they meet the pancreatic juice and are split, partly into short chains of a few amino-acids each, and partly entirely to free amino-acids. That the free amino-acids constitute a considerable part of the products of intestinal digestion was demonstrated by Abderhalden,[9] who isolated most of the known amino-acids from intestinal contents. That the entire mass of products, aside from the free amino-acids, consists of short chain peptides was shown by White and myself [10] with the nitrous acid method in the case of one of the lower animals, the dog fish. This work was done in 1910 at Woods Hole. Shortly after, London, by means of the formol titration, obtained results of the same nature with dogs.[11] Finally, either before or after entering the intestinal wall, the products encounter a third hydrolytic enzyme, erepsin, which is capable of completing the hydrolysis to the stage of amino-acids, in which form it appears that at least the greater part of the protein nitrogen is absorbed.

You will note that the above summary, which indicates the stage of our knowledge five years ago concerning the mechanism of protein nutrition, stops short against the intestinal wall. This was, as a matter of fact, the place where facts ceased and theories began. What happens to the amino-acids and peptides after they are absorbed from the intestine was not known. Neither amino-acids nor peptides could be detected in the blood. As the veteran Pflüger pointed out, the failure to detect either amino-

acids or peptides in the circulation might well be due to a lack of sufficiently delicate methods, for the flow of portal blood is so fast that even a maximum absorption of nitrogen might cause but a very small concentration in the blood at any given moment. Folin, in his classic paper on the theory of protein metabolism,[12] published eleven years ago, took the same stand. In order to fill the gap in experimental results, however, other authors proposed two theories. (1) The amino-acids are decomposed into ammonia and non-nitrogenous residues while passing the intestinal wall. (2) They are synthesized into blood protein. The latter theory, it will be noted, was particularly convenient, because it not only explained the failure to find amino-acids in the blood, but also gave the source of the blood proteins. It was especially championed by Abderhalden.

The development of adequate methods, however, showed that Pflüger and Folin were right, and both of the above explanatory theories became unnecessary. The first theory received its death blow at the hands of Folin. With the extremely delicate colorimetric method for the determination of ammonia which he devised, he was able to show that absorption of amino-acids from the intestine is accompanied by no increase whatever in the ammonia of the portal blood. When put to the rigid test of quantitative experiment, the deaminizing ability of the intestine vanished into thin air.

The fate of the resynthesis theory was similar. The sole foundation on which it rested was the negative results of attempts to find in the blood digestion products, either peptones or amino-acids. As soon as quantitative methods, namely, the formol titration and the nitrous acid method, were applied, however, it was shown by investigators working independently with each that the blood does contain amino-acids, and that they increase markedly during digestion. This was shown by Delaunay, working in Bordeaux, with the formol method, and with the nitrous acid method by Meyer and myself in the Rockefeller Institute. The normal amino-acid concentration in the blood of both dogs and men is near that of sugar, about 0.1 per cent., and it may be nearly doubled in the portal blood as a result of a heavy protein meal.

The force of these results was further strengthened by Abel, Rowntree, and Turner in their remarkable experiments with vividiffusion.[16] These experimenters passed the blood of living dogs through collodion tubes immersed in salt solution into which the non-colloid substances of the blood diffused. From the diffusing substances thus obtained they were able to separate in pure condition and identify several of the individual amino-acids. Abderhalden then also applied dialysis to blood and was able to obtain most of the amino-acids in sufficient amounts to identify them.

After entering the circulation, amino-acids disappear from it again very quickly. Within five minutes after 12 grams of alanine had been injected into the vein of a dog, 90 per cent. had disappeared from the circulation. A similarly rapid removal must occur during digestion, otherwise amino-acids would accumulate in much larger amounts in the blood than we observe. The question then naturally raised itself: What becomes of the amino-acids when they vanish from the circulation? Are they decomposed in the blood; are they at once somewhere synthesized into new protein; are they chemically incorporated into the complex molecules of the tissue protein; or are they merely absorbed by the tissues in general or by certain tissues in particular without undergoing any immediate change?

Analysis of the tissues of dogs which had received intravenous injections of known amounts of amino-acids answered these questions in favor of physical absorption.[17] In one experiment, which will serve as an example, the amount of amino nitrogen injected in the form of hydrolyzed casein was sufficient, if distributed evenly throughout the body, to raise the average amino nitrogen content of the tissues 40 mg. per 100 grams of tissue. The increases actually noted were: Muscles, 27 mg.; liver, 60 mg.; kidney, 60 mg.; intestinal wall, 50 mg. That the absorbed amino-acids could not have been in even loose chemical combination in the tissues was shown by the fact that they could be extracted by such mild agents as water, hot or cold, or dilute alcohol. They must have been held merely by physical forces.

The tissues, despite the great rapidity with which they absorbed amino-acids from the blood, never removed them from

it completely. An equilibrium is reached when, stated very roughly, the tissues contain about ten times the percentage of amino-acid present in the blood. From the fact that they are so much more concentrated in the tissues than in blood it is evident that the process by which they are picked out of the circulation is not a mere diffusion. If it were, we should find approximately equal concentration in both tissues and blood. The physical process, by which the exchange between blood and tissues is carried out, has not yet been definitely classified with any of the physical or physicochemical phenomena with which we are familiar. Until it is explained by such classification we cover our ignorance of the real nature of the process by giving it the general name of "absorption."

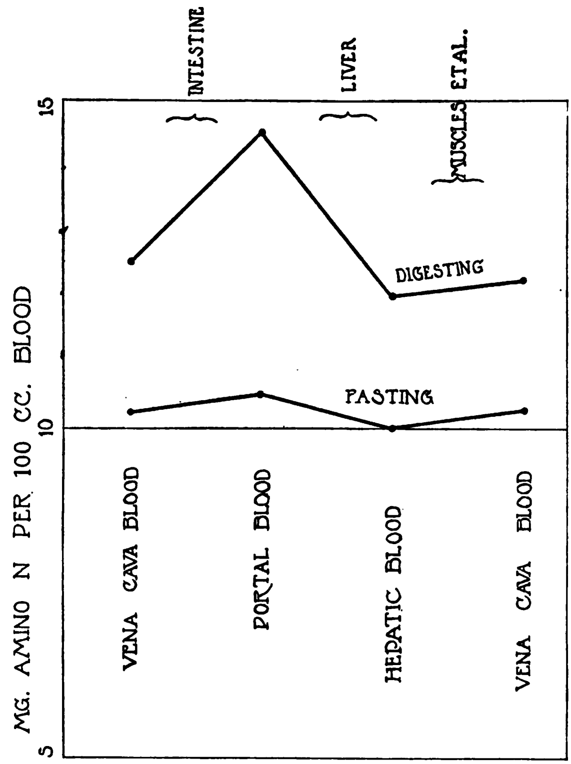
We have now followed the protein digestion products, that is, amino-acids, from the alimentary tract past the wall of the intestine into the blood stream and from the blood stream into the tissues. But we have yet reached only a temporary stopping place. Most of the protein nitrogen in the daily diet of an adult is excreted within twenty-four hours as urea; and Levene and Kober [18] found that when single amino-acids were fed to dogs they were excreted entirely as urea. It is evident that whatever stopping place the greater part of these products finds in the tissues is only a temporary refuge preliminary to their speedy destruction and elimination.

Present knowledge points to the liver as the organ which is most active both in absorbing amino-acids from the blood stream during normal digestion and in submitting them to the preliminary chemical alterations which precede elimination as urea or storage as reserve protein. Chart 4 shows that during digestion there is a greater fall in amino nitrogen during the passage of the liver (difference between portal and hepatic blood) than during passage through the entire remainder of the body (difference between arterial and vena cava blood). The liver is the organ to which the portal blood comes with its newly-acquired amino-acids and it is the liver that takes the lion's share of them. It follows as a necessary corollary that the liver must either store immense amounts of them after a heavy protein meal, or must

quickly transform them, either into urea for elimination, or into reserve protein for storage.

Further experiments have shown that the liver does not store amino-acids as such to an appreciable extent. Chemical trans-

CHART IV



The amino-acid content of the blood during fasting and protein digestion. Average of results from six fasting and six digesting dogs.

formation follows very quickly after absorption. This was shown in three different ways.

First, the tissues of dogs in fasting condition were compared in respect to their amino-acid content with those of dogs which were digesting or had digested large amounts of protein. It

was found that neither the livers nor other tissues of the fed animals contained a definitely greater store of amino-acids than did the tissues of the fasting animals. The digesting dogs must have either destroyed or condensed into protein all the amino-acids which they absorbed, and have done so at a rate which was practically parallel with that of absorption.

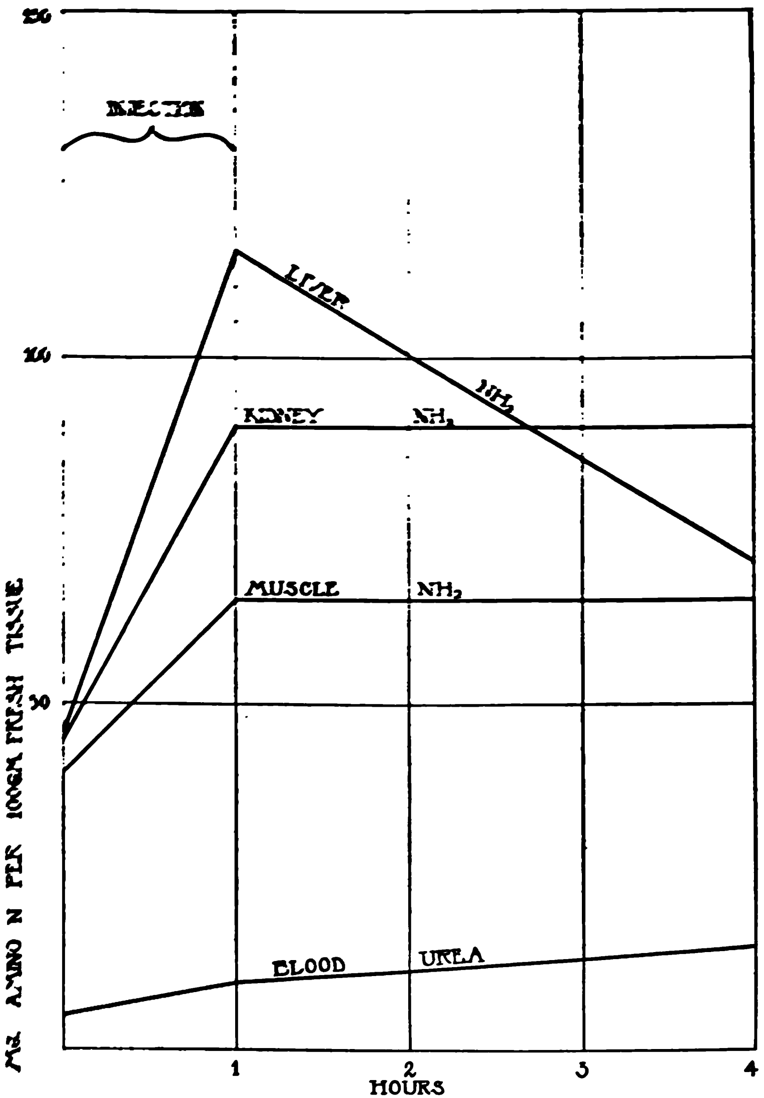
Second (Chart 5), dogs were injected intravenously with such amounts of amino-acids that the amino nitrogen content of all the tissues was raised considerably. Samples of muscular tissue, a lobe of the liver, and a kidney, were taken immediately after the injection, and again three or four hours later, the animals being kept under ether anæsthesia by the Meltzer-Auer insufflation method. It was found that, whereas the muscles and kidney still held after four hours all the amino-acids which they had absorbed, those taken up by the liver had disappeared. They had not been excreted, and there was no reason for assuming that they had been transferred to any other organ. The disappearance of the liver amino-acids was accompanied by a rise in the blood urea. The conclusion seemed justified, that the liver can destroy amino-acids at a rate very much greater than the muscles, and that at least a portion of the nitrogen of the amino-acids disappearing from the liver is converted into urea.

Third, comparison of Charts 4 and 6 shows that the blood in passing through the liver takes from it about as much nitrogen in the form of urea as it gives to it in the form of amino-acids.

All the above experiments emphasize the activity of the liver in metabolizing amino-acids, from which it produces urea as apparently the most abundant product.

On the other hand, it does not appear that urea formation is a process entirely confined to the liver. Folin's collaborators, Fiske and Sumner, have observed an increase in the blood urea when the liver was eliminated from the circulation.[19] Pavlov and Nencki in 1893 showed that a dog deprived of its liver could still form and excrete urea, though in decreased amounts.[20] It appears, therefore, that present experimental results may be interpreted by stating that the most active centre of amino-acid transformation, and of urea formation, appears to be the liver, but

CHART V

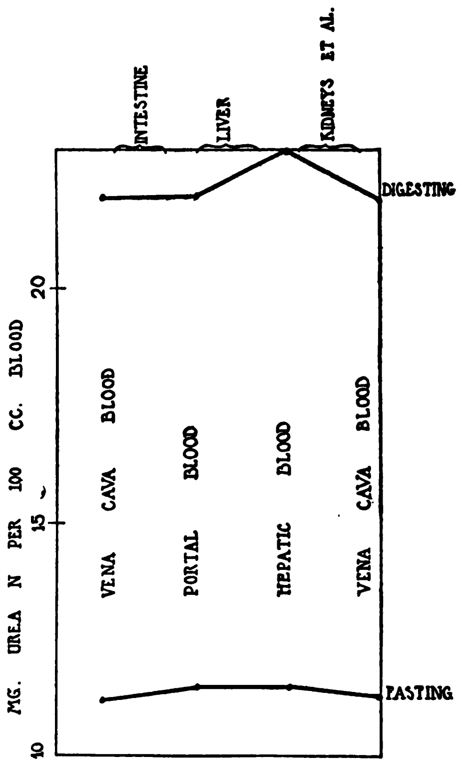


The absorption and retention, by different tissues, of amino-acids injected intravenously.

that the localization of the function is not absolute, and these processes also occur to some extent in other organs.

The next question to be raised is: Does the liver, during the digestion of a protein meal, wait till the other tissues are saturated with amino-acids, and then begin to destroy the unnecessary

CHART VI

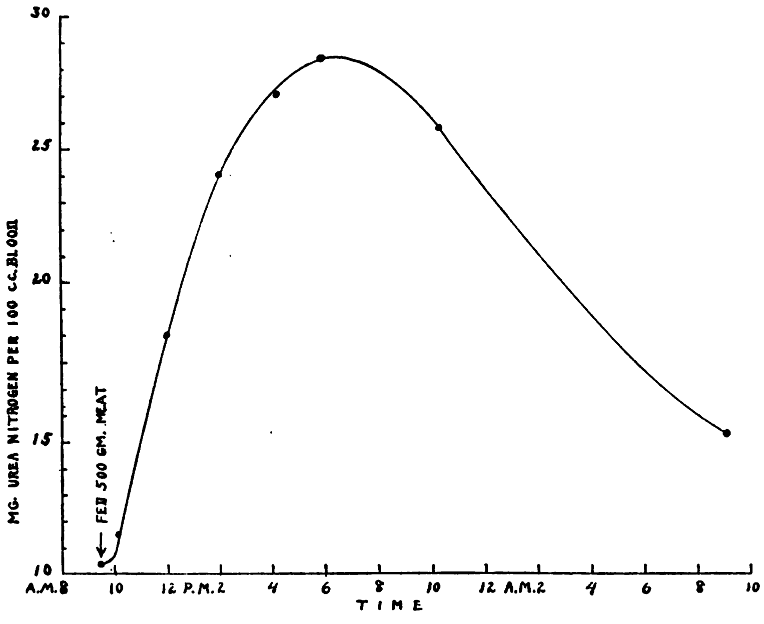


The blood urea during fasting and protein digestion. Average of results from six fasting and six digesting dogs.

excess, which is not needed by the organism, or does it begin to destroy the first that reach it in the portal blood? Unreasonable as it may seem, the latter behavior is what we observed. In order to test this point urea determinations were made at short intervals on blood from dogs which, after a two days' fast, had received

heavy meals of meat. It was found in all cases that the urea began to rise almost immediately after the meat was consumed. There was no interval of waiting commensurate with what might be expected if urea formation were delayed until after the tissues in general had replenished their store of amino-acids. It was furthermore shown by X-ray pictures that the blood urea began to rise at almost the minute the first particle of food passed

CHART VII



Time curve of blood urea changes during protein digestion.

from the stomach into the duodenum. Since London has shown that no absorption occurs until the chyme enters the intestine, our results indicate that the very advance guard of amino-acids entering the blood after a protein meal is, in part at least, immediately turned into urea. The interval between feeding and the beginning of urea formation is so short that this conclusion would really be forced upon us, even without the X-ray evidence. Un-

reasonable as it appears, the organism does not wait until it has absorbed sufficient protein digestion products to meet its immediate requirements, and thereafter begin to turn the surplus into urea. The very beginning of absorption stimulates the urea forming function into activity. This behavior explains the fact that no matter how depleted by disease or hunger the tissues of an individual may be, the greater part of the protein nitrogen which he may subsequently consume is excreted as urea, only a small portion being retained to rebuild the wasted tissues.

That urea is the form taken by all of the amino-acid nitrogen which disappears in the liver does not absolutely follow from our results. Pflüger was of the opinion that the liver cells store reserve protein from the food just as they store reserve carbohydrate in the form of glycogen. Our results do not at present exclude this possibility. In order to do so, we should have to prove that the liver gives out as urea an amount of nitrogen exactly equal to that which it absorbs as amino-acids, and our experimental technic does not yet enable us to say whether or not this is the case. The analytical methods are adequate, but the fact that the amino-acids are held for a certain time before they are destroyed, and that the urea also may not pass instantly from the liver tissue to the hepatic vein, make the striking of an exact balance between amino-acid intake and urea outgo a matter of experimental difficulty which has not yet been overcome. The possibility therefore remains open, though certainly not proven, that some of the amino-acids may be converted by the liver into a form of reserve protein which is stored like glycogen.

A word as to the significance of the free amino-acids which are stored as such by the tissues. They normally amount to from 2 to 4 per cent. of the dry weight of the various organs and might be regarded as a form of reserve food. That they are so in the same sense as fat and glycogen is, however, not the case. Reserve food supplies disappear during a prolonged fast. This occurs with glycogen and fat. It does not occur, even to a slight extent, with the amino-acids. If anything, they are slightly more abundant in the tissues of a fasting animal than in those of

one in a state of normal nutrition.[21] I believe that the explanation is that the free amino-acids, in the tissues as in the blood, are merely transitory bodies in the building up and the breaking down by body proteins. That all the aminoacids in the muscles of a well-nourished animal may have been immediately derived from food proteins would be believable. When, however, after a fortnight's fasting we find an equal or greater supply of free amino-acids in the muscles, we must attribute the source in this case to autolysis of the visibly disappearing tissues themselves. Consequently, it does not appear that the store of free amino-acid in the body functions to a significant extent as a food reserve, since it can neither be increased by feeding nor depleted by fasting. The free amino-acids are there both in blood and tissues, because they are intermediate steps in the never-ending processes of the building up and the breaking down of living protein.

We may, perhaps, most easily summarize the facts which have been brought out by tracing an amino-acid through the body as follows: Entering the alimentary tract as part of a protein molecule, it is set free by digestive hydrolysis and passes into the portal blood stream. It may be at once picked up by the liver. and decomposed into urea, or perhaps synthesized into reserve protein. It may, however, pass by the liver and be absorbed from the blood by one of the other tissues. Here it may remain for a time before being incorporated into the tissue protein. The fact that a considerable store of amino-acids is always found in the tissues is proof that chemical incorporation does not instantly follow absorption from the blood stream. After a period of time, concerning the length of which we are absolutely ignorant, the tissue protein autolyzes, and the amino-acid returns to the depot of free amino-acids held by the tissue. From this depot it may pass back into the blood, be taken out by the liver, and destroyed. Or it may in some tissue be reincarnated into a new protein.

We have hitherto dealt with the physiology of the amino-acids without any recognition of the differences between the individual members of the family. Whether we are concerned with their

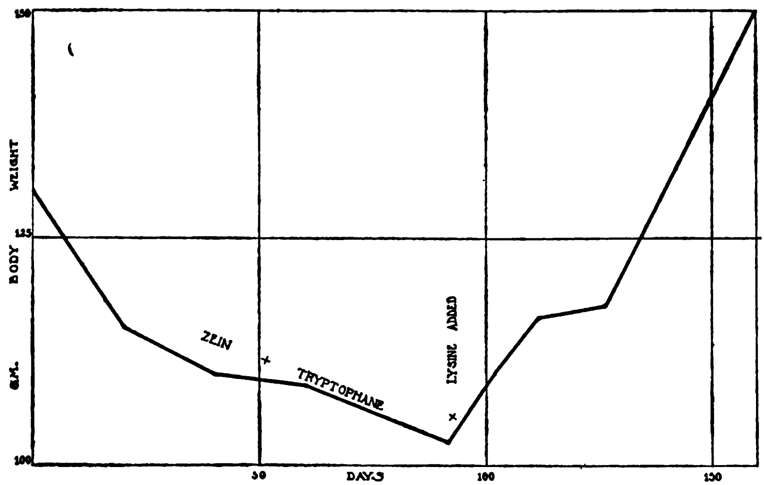
condensation into body proteins, or the manner in which, not being so condensed, they are destroyed, the individuality of the different amino-acids plays a most important rôle.

Let us consider briefly the indispensability of the different amino-acids for the nutrition of the body. All of the amino-acids which are known to occur in the native proteins enter into the structure of living protoplasm. The bacterium can synthesize them all from ammonia and sugar. Loeb has recently presented evidence that even lower animals, such as the banana fly, can also synthesize all of their amino-acids.[22] The higher animals can synthesize some, but must be supplied with others. One of the vital questions of physiology to-day is: which amino-acids can the mammalian body synthesize for itself, and which must be supplied ready-made to it in its food? The first even partial success in answering this question with experimental evidence was obtained by Hopkins of Cambridge, England.[23] He fed mice with food which contained as its sole nitrogenous constituent the corn protein zein. Zein contains no tryptophane. Eighty per cent. of the mice died within 20 days. When, however, tryptophane was added to the diet, only one-fifth of the mice died within 20 days, and most of them lived for over a month. Therefore it appeared, as has since been more rigidly proven, that tryptophane is one of the amino-acids which cannot be made in the body, but must be supplied in the food.

The study of the nutritional function of the individual amino-acids opened by Hopkins' pioneer investigation has been developed by our own chemists Osborne and Mendel who have studied the problem with a monumental attention to detail in the care, control, and even breeding of the rats used as experimental animals, in the accuracy with which the chemical composition of the food utilized was controlled, and in the wealth of experimental evidence with which point after point in the field has been settled. Professor Mendel himself has recently discussed the work before the Harvey Society, and it is mentioned here only because a paper on the physiology of the amino-acids would be incomplete without it. I have reproduced one of the hundreds of curves of

growth which Osborne and Mendel have published. This curve forms, in a way, a connecting link between Hopkins' work and theirs. It shows why the mice which Hopkins fed with zein plus tryptophane lived only a little longer than those which received zein alone. The rat whose weight curve is shown in Chart 8 received at first, like Hopkins' mice, a diet containing zein plus tryptophane as the sole nitrogenous constituents. During this period, although the animal was immature and should have been growing, his weight fell steadily. After 90 days another amino-

CHART VIII



Effect of adding lysine and tryptophane to diet deficient in these amino-acids.

acid, lysine, was added to the diet. Zein is lacking in lysine as well as tryptophane. The effect of making good both these deficits is shown by the immediate resumption of practically normal growth. Osborne and Mendel have proven beyond a doubt by such experiments that both lysine and tryptophane must be supplied to the higher animals in their food, since neither is synthesized in the animal body. A third amino-acid in the indispensable class is cystine, and metabolism experiments by Abderhalden indicate that tyrosine is a fourth.[25] That future

work will answer the question concerning the synthetic power of the body for other amino-acids may be expected with confidence.

That even the higher animals still retain the ability to synthesize the simplest of the amino-acids, glycocoll, is certain. The excretion of glycocoll can be stimulated by feeding benzoic acid. Instead of neutralizing it with ammonia, as it does with most other acids, the body condenses benzoic with glycocoll to form hippuric acid, in which form it is excreted. Magnus-Levy[26] found that by feeding rabbits large amounts of benzoic acid he could make them excrete more glycocoll in the form of hippuric acid than they possessed, either free or combined, in their entire bodies. This proved that they were able to manufacture glycocoll out of other nitrogenous substances. Osborne and Mendel have also found in their feeding experiments that glycocoll does not need to be fed in order to maintain growth, the rat being able to synthesize the amounts necessary for its growing tissue out of other nitrogenous substances. Whether any of the other amino-acids can be synthesized like glycocoll is uncertain. This field, the importance of which from both the practical and scientific standpoints is self-evident may be said to be still 13/18 virgin, since concerning 13 of the 18 amino-acids we have no conclusive knowledge as to whether we can synthesize them in our bodies or must depend upon plants to furnish them for us.

A discussion of the physiology of the amino-acids would not be complete without a word also concerning the manner in which those not incorporated into the body are broken down, yielding not only urea, but non-nitrogenous residuals which are burned or stored like fat or carbo-hydrate for their energy. The three men whose researches entitle them to speak with most authority in this field are Lusk, Dakin and Knoop. Knoop came from Freiburg three years ago to deliver a Harvey lecture on this subject, and, as our city is fortunate in claiming both Dakin and Lusk among its men of science, we either have heard or may reasonably hope to hear from them both the stories of their own researches. I will, therefore, attempt to indicate only in the most general way the manner in which the body is believed to dispose of its unincorpor-

ated amino-acids. The first step is the splitting off of the amino group, which yields ammonia and a hydroxy-acid, a hydroxyl group replacing the amino group of the amino-acid. The ammonia is turned into urea. The non-nitrogenous substance left after the amino group is split off is a fatty acid, and is dealt with accordingly. Varying with their structure, some of the amino-acids yield fatty acids which can be converted into glucose by the body, while others do not. Nearly the entire series of amino-acids has been tested in this respect by either Lusk or Dakin. The substances were either fed to phloridzinized dogs, whose urine was then analyzed for glucose, into which they turn everything that is physiologically capable of being turned into glucose; or the amino-acids were perfused through surviving livers, and the perfusion fluid was analyzed for glucose. The results are indicated by the plus and minus signs on the line at the bottom of Chart 3. The fact that half the amino-acids are glucose formers explains why diabetics can form sugar from protein as well as from carbohydrate. The fact that acetone bodies are formed from several amino-acids explains why diabetics may develop acidosis on a protein diet, or even when living on the proteins of their own tissues.

The nature of the fatty acid radicals left when the amino groups are removed from the amino-acids is also used by Lusk to explain the specific dynamic action of the proteins, their ability so to stimulate the metabolism that the rate of heat formation in the body is accelerated.[27] The amino-acids themselves cannot be responsible for this effect, because their concentration in the body is so well regulated, presumably by the liver, that no great fluctuations ordinarily occur, even after heavy consumption of protein. The stimulated heat production which Lusk and DuBois have discovered after the feeding of either protein or of amino-acids must therefore be due to their decomposition products, presumably the fatty acids that are formed by deamination; and the differences in the heat-stimulating effects of the different amino-acids are due to their individual differences, indicated by the varying shapes of their structural heads (see Chart 3).

PATHOLOGY OF THE AMINO-ACIDS

We now come to the significance of the amino-acids in pathology. The blood and urine have been investigated in regard to their amino-acid contents for the purpose of diagnosing or explaining pathological conditions which may be divided into two classes.

1. Those in which the normal function of catabolizing the amino-acids is injured. From the view that the liver is especially responsible for the conversion of the excess products of protein digestion into urea, it would logically follow that serious injury to this organ should result in a higher amino-acid content of the blood, and perhaps the urine. Consequently, amino-acids have been sought for by a number of investigators both as diagnostic indications and as toxic agents in liver atrophy, in conditions which involve visible injury to the liver, such as toxæmia of pregnancy, and in conditions which are presumably accompanied or caused by decreased liver function, of which diabetes is an example.

2. In the second type of abnormal condition in which amino-acid determinations have been called to the aid of the diagnostician specific ferments are supposed to be formed within the body which are capable of hydrolyzing tissues of an abnormal or pathological nature, thereby forming, either *in vivo* or *in vitro,* amino-acids from such tissues. The action of such specific ferments *in vitro* on the particular tissues towards which their activity is directed constitutes the Abderhalden reaction, which has been of late so largely in the public eye. Dr. Losee, of the Lying-In Hospital, Miss Vinograd, and I have devoted nearly a year of time to this reaction, and I shall consequently devote a moment to it here.

The Abderhalden reaction is based on the belief that when foreign proteins enter the blood stream the body cells elaborate and pour into the circulation enzymes which are capable of hydrolyzing the invading protein and none other. This idea was extended to include the proteins of abnormal tissues produced within the body itself. Thus, the epithelial cells of the placenta

of a pregnant woman are supposed to wander into the blood stream, and thereby stimulate the production of enzymes which can hydrolyze only the proteins of placenta tissue. Similarly, cancer cells are supposed to cause the production of enzymes capable of hydrolyzing only cancer tissue. The idea has been extended by various investigators to such an extent that, to judge from the claims made for the Abderhalden reaction, all that is necessary in order to settle a difficult diagnosis is to mix a little of the patient's serum with samples of tissue from all the suspected organs of the body, and the serum will infallibly pick out and digest the tissue from the affected part, leaving the other tissues unaltered. In justice to Abderhalden, it must be stated that his claims have never been so sweeping as those of some of his satellites. A great controversy arose over the Abderhalden reaction, some investigators reporting their results with enthusiasm, while others failed entirely, and still others took a middle course and utilized the customary "safety-first" formula, to the effect that there was evidently something in the reaction, but that results must be accepted with caution.

It appeared to us that the matter might be settled decisively if, instead of the rather uncertain color reaction with ninhydrin to detect the amino-acids resulting from digestion of the specific tissue, an accurate quantitative method were applied. And the nitrous acid reaction, because of its combined accuracy and specificity for amino groups, seemed to offer such a method. After preliminary experiments to ascertain the most satisfactory way in which to apply it, we finally settled on the following simple technic: Two c.c. of serum are incubated with placenta, as described by Abderhalden, and the undigested proteins are then removed by precipitation with colloidal iron. A control portion of serum is incubated and precipitated in the same way, but without placenta. The amino nitrogen is then determined in both filtrates. The increase in the nitrogen gas from the serum plus placenta over the nitrogen from serum alone indicates the extent of digestion that has taken place. The results could be obtained with great accuracy, and the increases observed were many times larger than the experimental

error. Consequently we believe that we were successful in excluding such error as a factor in interpreting the results. In order to give the reaction the fairest test possible we utilized it only as a test for pregnancy, and the non-pregnant controls were normal men and women, hospital patients. We made several hundred analyses. Both normal and pregnant sera showed a measurable amount of digestive activity, and the results with both varied over practically the same range. A slightly higher average obtained with pregnant sera may explain the fact that some honest investigators have been led to believe that if they could eliminate their own errors they would find the reaction all that had been claimed for it. But even the difference in averages was not significant, and the individual results from perfectly normal subjects covered practically the same range as those from pregnant women.[28] Entirely similar results were obtained by Isaac Levin and myself in attempting to apply the reaction to cancer diagnosis.[29]

We come finally to attempts to detect by amino-acid determination conditions involving impaired liver function. That tyrosine may be found in the urine in acute yellow atrophy is the classical fact in this field. The more the problem is studied with quantitative methods, the more it appears, however, that the liver injury must be extreme before it can cause unusual accumulation or excretion of amino-acids. Soon after the nitrous acid method was perfected I determined the amino nitrogen in the urines of dogs which Drs. Dochez and Opie had treated with chloroform and phosphorus, and was greatly surprised to find no increase in the percentage of amino nitrogen, despite the fact that autopsy showed extreme liver degeneration. More recently Marshall and Rowntree have found that the urine of such dogs, if taken immediately before death occurs, does show an increase in amino nitrogen, and that a still greater increase occurs at this time in the blood.[30] It is evident, however, that, in dogs at least, the liver injury must be most severe in order to affect the amino-acid content of the blood or urine. That it must be equally severe in man does not necessarily follow. Chesney, Marshall and Rown-

tree report that a considerable proportion of patients with impaired liver function showed abnormally high amino-acid nitrogen in the blood.[31] Consequently, although it cannot be said that amino nitrogen of either blood or urine offers at present much assistance to the diagnostician of diseased livers, it may be possible that the very constancy of the amino nitrogen figure under most conditions will enhance its diagnostic value for such conditions as do affect it.

That advanced diabetes is such a condition has been claimed by Cammidge.[32] According to his view in the milder stages of diabetes the body partially loses its ability to burn glucose, but it can still transform amino-acids into glucose. In the most severe stage, however, it cannot even accomplish the prelimniary transformation of amino-acids into glucose, but excretes large amounts of them unchanged. We have performed determinations of amino nitrogen in both blood and urine on a considerable number of patients in Dr. Allen's diabetic clinic at the Rockefeller Hospital, and have found that urines from certain of the patients do show figures distinctly higher than normal. That the high figures indicate diabetes of a special gravity, however, we are not yet prepared to state.

We finally come to the toxæmias of pregnancy. Ewing and Wolf[33] some years ago showed that the urines of such cases had a decreased proportion of urea nitrogen, and an increase in the undetermined nitrogen. From this, and from the gross injuries which the liver suffers during the toxæmia, Ewing and Wolf suggested that the intoxication might be due to protein digestion products which the degenerated liver could not metabolize, and which caused both the toxic symptoms and the increase in the undetermined nitrogen of the urine. The methods used for urine analysis were the most complete available at the time, and the hypothesis put forward was certainly reasonable. However, Dr. Losee and I, in examining both urine and blood from a considerable number of patients with toxæmia of pregnancy, have found in no instance that either blood or urine showed an abnormal concentration of amino-acids or of intermediate protein digestion products. Consequently, the responsibility for the toxæmias of pregnancy cannot be left with the amino-acids. I may add that

we have also tested the hypothesis that acidosis is to blame, with essentially negative results. We must frankly face the fact that we are entirely ignorant concerning the chemical nature of the substances which cause these toxæmias.

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SPIROCHÆTES *

DR. HIDEYO NOGUCHI
The Rockefeller Institute for Medical Research

TO-DAY a spirally shaped micro-organism may be called either spirochæta, spirillum, treponema, spironema, cristispira or saprospira, according to the characteristics of the organism. The choice of the generic name for a given variety is still very much dependent upon the individual views held by different investigators, and this has led to a somewhat chaotic state of affairs in the nomenclature of this group of organisms. This is found to be the case more especially in the medical literature where these minute spiral organisms play an important part as causative agents of certain diseases. Nevertheless, thus far but little attention has been paid to the systematic position occupied by them. Since Ehrenberg[1] in 1838 introduced a new generic term "Spirochæta" to designate a free living spiral organism which he found in a swamp near Berlin, it remained practically unnoticed until 1904, when Schaudinn[2] stated as his view that certain spirochætes constitute a phase of the life cycle of trypanosomes; hence, that they are of protozoan origin instead of being plants. It may here be mentioned that Ehrenberg, Migula, and other systematists classified Spirochæta under bacteria, which classification was accepted for nearly seventy years. Indeed, it was not uncommon among medical authorities to employ the terms Spirochæta and Spirillum interchangeably. Medical men may consider the causative agent of relapsing fever as being either a Spirillum or a Spirochæta, according to their inclination. This sort of indiscriminate use of terms has gradually extended to other spiral organisms, such as the causative agent of syphilis. According to the old school it was of very little importance whether a spiral organism had one or two polar

* Delivered February 5, 1916.

flagella or a tuft of flagella, so long as both Spirochæta and Spirillum belonged to the same family. On the other hand, Schaudinn and his school maintained that the difference between Spirochæta and Spirillum is no longer so easily disposed of, since one is of plant and the other of animal origin. The revolutionary view of Schaudinn was based chiefly upon his observations on a protozoan, Leukocytozoon ziemanni, found in the blood of the Little Owl (Athene noctua), and regarded by Schaudinn as a trypanosome, which is said to undergo a spirochætal stage while passing through an intermediary host (Culex pipiens). While the accuracy of Schaudinn's observations has been questioned by later investigators,[3, 4, 5] the great impetus which his theory occasioned has had a far-reaching effect upon the development of our present knowledge concerning the organisms generally known as "spirochætes." It was soon after announcing his views that Schaudinn made his famous discovery of the occurrence of Spirochæta pallida in syphilis. In their first publication Schaudinn and Hoffmann[6] gave the name Spirochæta pallida to the spiral organism found in syphilitic lesions because of its resemblance to spirochætes in general, but within a year Schaudinn recognized certain features (preformed cylindrical spiral filament, difficulty in staining, regularity of curves, etc.) which he considered distinctive enough to classify it apart from the usual spirochætes (changeable curves, taking on of a violet component of Giemsa, no preformed spiral, ribbon form, etc.).[7,8] Thereupon he replaced the generic name Spirochæta with a new term Treponema.[9] This all occurred in 1905. But before Schaudinn had had time to decide upon a new generic name for his organism, Vuillemin[10] (1905) proposed that it be called Spironema. In the meanwhile some authors, particularly in France, commenced to use the term "spirilla." There were also some newer generic names created by still later systematists; for example, Microspironema[11] (Stiles and Pfender*), Borrelia[12] (Swellengrebel), Spiroschaudinnia[13] (Sambon), and Spiro-

* The statement of some authors (Gross and Gonder) that this antedated Schaudinn is erroneous. Schaudinn published his note on Oct. 19, and Stiles and Pfender on Dec. 2 of the same year.

soma [14] (Schilling), but these are of no importance to-day. The only difficulty in choosing the generic name for "Spirochæta pallida" lies in the fact that although Schaudinn corrected his error within several months after his discovery another suggestion had meanwhile been made to answer the same purpose, and according to the international code of nomenclature Vuillemin's Spironema would have had to receive preference over Schaudinn's own Treponema, had it not been for the fact that the term Spironema as proposed by Vuillemin is not acceptable to those who maintain, like Schaudinn, that the organism of syphilis belongs to the Protozoa, because in 1892 it was used by Klebs as a genus of Flagellate.[15] The same name had also been used by Meek in 1864 for a fossil snail. Of course, "Spironema" may be available for any one who holds that "spirochætes" do not belong to Protozoa.* Thus, Gross [16] (1910) used this term to include various spirochætes allied to the spirochætes of relapsing fevers, syphilis, etc., with the specification that he believed these to be of a bacterial nature. It may be mentioned that the term "Spirochæta," as taken up by Schaudinn in 1905 in the sense of protozoan organism, had already been used by Michael Sars in 1856 for an annilid genus. It seems that the creation by Schaudinn of the genus Treponema was perfectly justified, although not all the characteristics attributed by him to this genus are found to be distinctive from those of other "spirochætes." Schaudinn did not live long enough to witness the gradual modification which the Spirochæta question went through. As a result of the works of various systematists and zoologists, we are brought to realize that the original S. plicatilis, described by Ehrenberg in 1838, is an entirely distinct organism and bears little relation to the other organisms which we now call "spirochætes." We also know that the latter should no longer be designated as spirochætes, and that the spiral organisms found in the crystalline style of various mussels are neither trypanosomes, as held by Perrin [17] nor typical

* The use of two identical terms, one in the animal and the other in the plant kingdom, has been known to occur and is permissible. For example, "Bacillus" and "Coccus" are found in zoölogical as well as in botanical genera.

spirochætes, but form another group which may be seen to possess one or more genera. These facts were revealed after the death of Schaudinn by the careful studies of Novy and Knapp,[18] Schellack,[19] Gross,[20, 21] Zuelzer,[22] Gonder,[23] Dobell,[24, 25, 26] Hoelling,[27, 28] Fantham,[29, 30] Swellengrebel,[31] Bosanquet,[32, 33] and others. Although much light has been thrown upon the structure of these organisms, no definite conclusion has yet been reached as to the affinity of the "spirochætes" in the system of natural history. While there are still some who consider "spirochætes" as allied to bacteria and others who regard them as of a protozoan nature, there now appear to be certain authors who are inclined to set them apart both from bacteria or protozoa and to place them in the domain of the Protista, *i.e.*, organisms belonging to neither plant nor animal. Dobell [24] represents this view, and Dofflein [34] compromises by calling them Proflagellates, and placing them between Bacteria and Protozoa. Zuelzer [22] holds a somewhat similar opinion to that of Dobell. In order to bring up some of the more important data relative to the question of classification, we shall now review the present situation.

As remarked at the beginning of this paper the Spirochæta of Ehrenberg was regarded as a genus of the family of Spirillacea and no question was raised in regard to its possible affinity with the Protozoa until the publication of Schaudinn's fascinating observations on Leukocytozoön ziemanni. Since that time there have appeared numerous partisans of Schaudinn's view that so-called spirochætes are of protozoan nature. Their main contentions are based on the following characteristics: (1) Longitudinal division as the mode of multiplication; (2) presence of an undulating membrane; (3) high degree of bodily flexibility; (4) absence of cell membrane; (5) absence of a motor organ such as the flagella; (6) presence of a periplastic process; (7) peculiar nuclear arrangements; (8) band-like bodies; (9) encystation or resistant form; (10) a certain periodicity in their pathogenic activity in the infected hosts; and, (11) effect of certain chemicals such as sodium taurocholate, saponin, etc., which bring about the dissolution of these spiral organisms and thus offer a contrast to the great resistance shown by bacteria (especially spirillum) to these substances. The foregoing characteristics tended to place the spirochætes in the Flagellate group, but subsequent studies by different investigators, especially those who have employed a more recent and approved cytological technic, seem to indicate that many of the above criteria were based upon erroneous or insufficient observations. According to the observations of Dobell,[34]

Gross.[14, 20, 21] Zuelzer,[22] Swellengrebel,[20] Novy and Knapp,[23] and others, the following features are characteristic of "sphirochætes."

1. In the case of the majority of "spirochætes" transverse division is the only mode of multiplication (Koch. Levaditi, Fraenkel, Novy and Knapp, Borrel, Gross, Zuelzer, Swellengrebel, Schellack, etc.). Only in certain pathogenic small varieties has the occurrence of longitudinal division been reported.[7, 25]

2. No undulating membrane has been definitely demonstrated in any spirochæta. The alleged undulating membrane depicted by Perrin[32] and Schaudinn[34] in the dried preparations of certain mussel spirochætes is an artefact brought about by improper fixation, namely by the torn crista of a cristispira.[35]

3. The alleged chromatin rods and spirals described by Perrin in the case of certain mussel spirochætes known as Spirochæta balbianii (Cristispira) are now said to be nothing but a distorted arrangement of volutin substance or chromidial granules which under optimum fixation gather themselves along the walls of the chambered structure of the cell body.

4. The absence or presence of cell membrane seems to depend upon the variety of "spirochætes." Thus, the original type organism of Ehrenberg was described as being devoid of a membrane and is still so regarded by all who have studied this organism. On the other hand the mussel spirochætes and various small parasitic species are now said to be provided with a thin but elastic membrane which cannot be differentiated from the cell body by means of staining reactions. The presence of a membrane would suggest a close affinity with Spirillum, but the latter has a stiff non-elastic membrane.[33]

5. In regard to the motor organ no generalization can be made. The original type organism of Spirochæta and all mussel spirochætes are devoid of any motor apparatus. On the other hand, a terminal process, consisting of a delicate, elastic filament with minute, regularly set curves, may in the case of various small parasitic spirochætes be found to project from one or both ends of the body. Borrel[37] and Zettnow[38] obtained some preparations in which the Spirochæta of fowl spirillosis and relapsing fever appeared to possess peritrichial flagella, but this must have been a case of artefact formation as no one has since been able to confirm their findings. Schaudinn considered the terminal process to be identical with the periplastic appendage of a flagellate.

6. Certain spirochætes such as S. balanitidis and S. buccalis, and others, were said by Schaudinn,[36] Hoffmann and Prowazek,[39] to have a flattened, ribbon-formed body. Later investigations hold that the body is cylindrical and round on section.

7. Encystment, or the resting stage, such as observed in protozoan organisms, has been suggested[37, 40] as existing, but never satisfactorily proved.

It will be seen that the findings of later investigators deduct much of the foundation upon which the protozoan theory of "spirochætes" had been based. Not only do they separate the spirochætes from the Protozoa, but they also bring out certain new facts which make it difficult to include them among the Bacteria as was formerly done by those who opposed the view of their protozoan nature. As has been briefly remarked, the spiral organisms called spirochætes are not of uniform structure, but, according to recent investigations, fall under several great divisions. It was owing to the imperfection of the methods of study that the free living forms and numerous parasitic varieties were at one time all held to belong to the same genus. Since the introduction of dark-field microscopy [41] many points which could not be satisfactorily determined with stained specimens have been carefully checked up and the entrance into the field of certain excellent cytologists has helped to clear up many points relating to the systematic grouping of these organisms. These cytologists made extensive series of comparative studies, at the same time carefully examining the structure of bacteria, spirilla, spirulina, and oscillaria.

As has been pointed out by Bütschli, [42] [43] bacteria are composed of a central body and a plasmatic layer. The former contains volutin granules and some chromidial elements. The spirillum has a series of chambers, each of which is constructed like a single bacterial cell. Both are covered with a stiff cell membrane. The structure of Spirulina is similar to that of Spirillum, differing from the latter by the highly flexible character of the membrane. Now, a very similar structure was demonstrated by Gross [20] in the body of mussel spirochætes and speedily confirmed by Dobell, [24] Zuelzer [22] and others. Gross, Dobell and Zuelzer all agree that the original free-living Spirochæta described by Ehrenberg is a unicellular organism which bears no relation either to the mussel spirochætes or to the small parasitic varieties. This fact implies the dissociation of the long used term "Spirochæta" from those organisms which in reality were commonly known as spirochætes. Odd as it may seem, the true Spirochæta has been but rarely studied, even by biologists, and certainly not to any great extent by medical men who have so much to do with the so-called "spirochætes."

Gross [23] was the first person who proposed to distinguish the true spirochæta from the other varieties of spirochætes by creating new genera for the latter which, according to his studies, could not be classified with spirochæta in the strict sense of the term. Thus for the latter type he created the name Cristispira (those with Crista), for the large parasitic spirochætes in fresh shell fish, saprospira (those without Crista), and the small parasitic varieties, including all pathogenic species, he designated as Spironema. Gross maintains that Cristispira, Saprospira and Spironema belong to the bacteria and places them under the family name of Spironemaceæ. Gross and Bosanquet recorded a few instances in which certain mussel spirochætes went into sporulation comparable to the true bacterial feature.

Dobell and Zuelzer both admit the striking resemblance between the chambered structure of Spirillum and Cristispira, but cautiously avoid accepting the bacterial theory of Gross on the ground that the last-named organisms have a more elastic and flexible membrane and that they are not necessarily bacteria. Dobell, as has been stated, has proposed a new family name Spirochætoidea which should include not only Gross's Spironemaceæ but also Spirochæta. The writer does not accept Gross's Spironema as it was applied to a flagellate in 1892 (Klebs), but retains Schaudinn's Treponema to designate all small parasitic and pathogenic varieties. He does not consider that there is a sufficiently essential difference between them to warrant two genera. Zuelzer regards the affinity between the mussel spirochætes and spironema (one of the Cyanophyceæ genera) as being much closer than that between these types and spirillum. On the other hand, Gonder accepts the classification of Gross more completely. He does not, however, share Gross's view that these organisms are definitely of a plant nature, holding that certain features indicate their partial affinity to the protozoa. He also differs from Gross in retaining Schaudinn's term Treponema for the organisms of syphilis and yaws and such affections, while accepting Gross's term Spironema for other varieties such as the spirochætes of relapsing fevers, tick fever, etc. The situation is still confused.

Classification After Gonder (1912)

SPIRONEMACEA (GROSS, 1910)

Spirochæta (Ehrenberg, 1838)	Type: Spirochæta plicatilis, etc., all free living.
Cristispira (Gross, 1910)	Type: Cristispira balbianii, and other varieties found in mussels.
Spironema (Vuillemin, 1905)	Type: Spironema recurrentis, and other parasitic and pathogenic varieties living in blood.
Treponema (Schaudinn, 1905)	Type Treponema pallidum, Treponema pertenue, and other varieties with closely set spirals.

Classification After Gross (1912)

Spirochæta (Ehrenberg)	Type: Spirochæta plicatilis. Unicellular organism without a membrane or flagellum, highly flexible. Free living. Transverse division.

Spironemacea (Gross, 1912)

Cristispira (Gross, 1910–11)	Including different varieties living in certain mussels. C. balbianii, C. anodontæ, C. pectinis, etc. All possess a crista. Chambered structure of the body. Sporulation. Transverse division.

Saprospira (Gross, 1911)	Similar to the foregoing except that there is no crista. Found in foraminiferous sand. Sporulation. Transverse division.
Spironema (Vuillemin, 1905)	Including small parasitic varieties: S. pallidum, S. pertenue, S. recurrentis, S. gallinarum, etc. Probably multicellular (or chambered). Transverse division. Flagella or terminal thread present.

Classification After Dobell
spirochætoidea (dobell) 1910–1911

Spirochæta (Ehrenberg, 1838)	Free living forms, fresh water or marine. Spirochæta plicatilis (Ehrenberg) Sp. gigantea.
Treponema (Schaudinn, 1905)	Parasitic in animals, vertebrates and invertebrates. T. pallidum (Schaudinn), T. recurrentis, T. dentium, etc.
Cristispira (Gross, 1910)	Parasite in Lamellibranchiata (mussels). C. balbianii certes, C. anodontæ, C. pectinis, C. veneris.

Classification After Migula (1897)

Bacteria	Coccaceæ, Bacteriaceæ, Spirillaceæ, Chlamydobacteriaceæ and Beggiatoaceæ.

spirillaceæ

Spirosoma	Rigid; no organ of motion.
Microspira	Rigid; one seldom two or three, polar wavy flagella.
Spirillum	Rigid; polar tufts of 5–20 flagella, mostly semicircular or wavy.
Spirochæta	Flexuous, motion organ unknown, probably an undulating membrane.

Classification After Swellengrebel

Bacteria	Spirochætaceæ	More or less distinct properties of protozoa but not much more so than the bacteria capable of forming S or Fe in contrast to those producing nitrification. Plasmolysable like the Spirilla.
	Spirillaceæ	
	Coccaceæ	

Classification After Levaditi (1912)

Spirillaceæ (Migula)

- 1. Sub-family, Spirillacea (Swellengrebel). Not flexible.
  - 1. Genus, Spirillum.
  - 2. Genus, Vibrio.
- 2. Sub-family, Spirochætacea. Spirally flexible, surrounded by periplastic cover, with or without flagellum-like appendage.
  - 1. Genus, Spirochæta. No preformed spiral; loss of spiral form at rest.
    - a. No flagellum with undulating membrane.
    - b. Flagellum, but no undulating membrane.
      - One or two terminal flagella. . . . S. buccalis. S. balanitidis. S. refringens.
      - Peritrichial flagella. . . . S. gallinarum. S. duttoni. S. obermeieri (?)
  - 2. Genus, Treponema. Preformed spiral; no visible undulating membrane.
    - a. Flagellum at one end. . . . . T. pallidum. T. framboesia.
    - b. Flagellum at each end. . . . . S. dentium.

Let us consider each of the groups in some detail on the basis of a newer classification.

Spirochæta.—According to Schaudinn [36] the type organism of Spirochæta possesses certain features which are also found in trypanosomes—an undulating membrane, periplastic fibrillar process, longitudinal division, etc. But this apparent resemblance has been shown to be erroneous. Thus, according to the latest contributions made by Zuelzer,[22] the original type organism, Spirochæta plicatilis, has no chambered structure, but is provided with a straight fibrillar axial filament surrounded by a plasmatic spiral layer which covers it unequally in different places. The organism consists of a single cell. Volutin granules which can be demonstrated by certain microchemical reactions are regularly disposed within the plasmatic layer. During motion the plasmatic layer at a given position becomes thickened or reduced in volume according to the current of the substance. The spirals of the plasmatic layer surrounding the straight axial filament occur regularly and closely, while the whole body shows several irregular undulations. There is no flagellum or periplastic terminal process, and no membrane has been demonstrated. It measures 100–200μ on an average, sometimes attaining a length of 500μ, whereas it is only 0.5–0.75μ in width. Unlike the other spiral organisms bearing the name of spirochæta (undoubtedly indiscriminately applied) the members of this group of real Spirochætes do not swim, but their locomotion is effected by a creeping movement along the surface of a supporting object. Multiplication is brought about by transverse division which is effected by a thickening of a certain part of the axial filament where a cross fissure takes place, followed by the strangulation of the plasmatic layer at the corresponding spot. Since Ehrenberg described the first species, four more species have been added, one by Cantacuzène in 1910,[46] and three by Zuelzer in 1912.[22] They are all free living and are not known to be responsible for any pathological conditions in either human beings or animals.

Since the essential characteristics of the group of true Spirochæta do not agree with those of various other species hitherto unreservedly called spirochætes, the necessity of reclassification

became apparent as soon as these facts were known about 1910, whereupon Gross, Dobell and others undertook special studies in this connection. As has been mentioned, Gross, Dobell, and Gonder all possess their individual ways of classification, but all agree on one point, *i.e.*, that the majority of the organisms known as spirochætes are not spirochætes in the strict systematic sense and must, therefore, be differently designated. Gross was the first to do this and he was followed by Dobell and Gonder, who introduced some modifications, but it seems that the family term Spironemacea of Gross has found a wider acceptance than Dobell's Spirochætoidea, although both include practically the same constituent organisms under a slightly different generic name. Thus Dobell accepts Gross's generic names Cristispira and Saprospira (provided that this genus can be recognized by other investigators) to cover the varieties found in shellfish, while preferring to use Treponema instead of Spironema as proposed by Gross. Dobell's family Spirochætoidea comprehends, besides all the constituents of Gross's family Spironemaceæ, the genus of the true Spirochæta. Whether the segregation of Spirochæta from the other genera composing Spironemacea is justified or not seems still debatable, inasmuch as the differences between the genus Cristispira and the genus Spironema are, I believe, no less striking, and induced Gross to separate the Spirochæta from them. According to personal observations on small "spirochætes" there seem to exist more affinities in the structure of true Spirochæta and the small parasitic varieties than are assumed by Gross and other investigators. For the present I will dwell upon different groups of organisms which those investigators have classified as separate genera, and in order to give a basis for further development of the subject, I propose to employ the new generic names proposed by Gross without, however, committing myself to his views.

Cristispira.—This genus was created by Gross in 1910 for the large saprophytic commensal spiral organisms found in the alimentary canal of certain varieties of shellfish. They are chiefly found in the crystalline stile which is a jelly-like projection in the stomach. The most unique feature of the genus is the presence

of a crista or ridge which extends spirally along the whole length of the body, whence the name Cristispira. Certes [47] considered the type organism of the genus Cristispira balbianii to be a trypanosome on account of the presence of an undulating membrane (later recognized by Gross as a ridge) and it has been called Trypanosoma or Spirochæta indifferently. Laveran and Mesnil [48] in 1901 regarded it as allied to the bacteria. Perrin [17] in 1905–1906 took up the subject and arrived at the conclusion that it has many features in common with the trypanosomes. This he observed from stained preparations in which he found an undulating membrane, a spirally arranged nuclear rod, as well as various mitotic figures and longitudinal division. Perrin's observations were in part confirmed by Keysselitz, [49, 50] Swellengrebel,[31] Hoelling,[27, 28] Gonder,[45] and Fantham,[29] but a later investigation of Schellack [19] brought out an entirely different set of facts. According to Schellack the undulating membrane and spiral nuclear rod or alleged karyokinetic figures are an artefact caused by improper fixation (dry method). In properly fixed preparations the cell-body is composed of an alveolar protoplasm and contains a number of transverse walls. In their later works Gonder [45] and Fantham [29] confirmed Schellack's observations. Zuelzer [22] and Dobell found chromatin (and volutin) granules to be deposited along the surface of the transverse septa, while Gross [20] failed to see any chromatin granules in Cristispira. On the other hand, Hoelling thinks that the entire cell-body is saturated with diffuse chromatin substance. The chambered structure of the cell-body is regarded by Gross as a sign of the multicellular nature of the organism, but many authors hesitate to accept this view, maintaining that it is a single organism with numerous cross septa. Gross, Zuelzer and Dobell all agree that the cell-body is surrounded by a strong membrane similar to that found in bacteria, although Zuelzer distinguishes it from the latter by its high flexibility. They found that the membrane had a double contour and protected the cell-body from the solvent action of various substances, such as saponin, as well as from acids and alkalies, a fact explained by Gonder as not necessarily due to the presence of a membrane but to the more concentrated external

fibrillar layer on the cell surface. In fact, Gonder described a fibrillar appearance of the external layer of the cell-body after the organism had been acted upon for some time by certain chemicals.[45]

Opinions still vary as to the origin of the ridge or crista. Earlier workers viewed it as an undulating membrane.[17] Gross, Zuelzer and Dobell hold that it is a superposed structure having no direct connection with the cell-body, while Schellack regards it as a true periplast traversed by numerous fibrils. He believes that the so-called undulating membrane of the authors of the term Cristispira is an artefact produced by defective technic. Hoelling as well as Fantham and Porter [35] entertain a view similar to that of Schellack, and the presence of a myoneme in the periplast was even maintained by Fantham and Porter. Mackinon [51] and Vlès [52] were unable to demonstrate any myoneme in the periplast, although Borrel and Cernovedeanu [53] assume that there exists a myoneme in the membrane which enables it to flatten or fold the ridge. When the organism is subjected to macerating or solvent agents (saponin, acid, alkali, etc.), the membrane is first attacked. The delicate fibrils become quite distinct in the course of dissolution, but the whole structure finally disappears completely, showing the plasmatic nature of the membrane. The cell-body is much more resistant.

Division is exclusively transverse according to the investigations of Schellack, Gross, Zuelzer, Dobell, and Laveran and Mesnil, while earlier investigators (Perrin, Keysselitz, Gonder, etc.) considered it longitudinal. Fantham and Porter [35] working with S. obermeieri and S. duttoni found both modes of division to occur. It is possible that a peculiar mode of division, described by Gross [20, 54] as an *incurvation,* might have been the cause of mistaking it for longitudinal division. Incurvation is a phase of the transverse division of Cristispira, whose body first doubles up (incurvates) at the segment where the fission is to take place and then after some time completes the process. During the incurvation both halves of the organism intertwine and simulate a stage of longitudinal division.

Sporulation was described by Gross [44] who saw a Cristispira

produce a series of somewhat smaller, highly refractile, oval bodies out of the square chambered structure of the cell body. These oval bodies were seen to separate into individuals, but no new cristispira could be made to sprout out of these bodies (or so-called spores). Bosanquet [32] made a similar observation. The question of sporulation is still open to further confirmation and is very important in view of the divided opinion regarding the affinity of this group in the system.

The cell-body is highly flexible, round on section, wavy or spirally wound, possessing not more than three or four curves. There are neither flagella nor terminal projections, except in one small species, C. spiculifera, which Schellack described as having a terminal filament.

There are about 18 known species which inhabit different varieties of shellfish belonging to nearly twelve different genera of Lamellibranchs, including common oysters and fresh water mussels. These genera are Ostrea, Anodonta, Chama, Pinna, Mactra, Pecten, Modiola, Lima, Gastrochæna, Saxicava, Tapes and Umo. Cristispira balbianii and C. anodontæ are the largest species and measure 100–130μ in length and 3–5μ in width, while the smallest representative of the genus, C. papillosum, measures but 18.5–20μ by 1.1–1.4μ.

Saprospira.—Gross [20] proposed to introduce this genus in order to group together a new species of mussel "spirochætes" which distinguished themselves from Cristispira by the absence of a crista. Their habitat and other cytological features are the same as those noted in the Cristispiræ. According to this investigator, Saprospira grandis and S. nana undergo multiple transverse division and bear a more distinctly bacterial aspect.

Spironema and Treponema.—Under Spironema Gross classified all the pathogenic and small saprophytic varieties. Dobell [24] substituted Spironema for Schaudinn's Treponema on the basis that the former term was applied to a flagellate (Spironema multiciliatum,[15] by Klebs, in 1892; for he did not consider it necessary to create two genera out of these organisms. Gonder still hesitates to drop the distinction between the group of "blood-spirochætes" and that of "tissue-spirochætes," the latter con-

taining Treponema pallidum as type organism. While Schaudinn's original criteria for Treponema are no longer valid as regards several points, Gonder proposes to retain the term Treponema for the pallidum group and to accept Spironema for the more irregularly curved, wavy varieties to which most of the "blood-spirochætes" and saprophytic parasites belong. From personal observations I believe the differences between the two groups to be differences of degree, not of quality. They should belong to one and the same genus, as may be seen from the characteristics enumerated below. Spironema and Treponema have a slender, cylindrical, spirally wound, highly flexible cell-body, which exhibits serpentine, cork-screw-like, and sometimes lashing movements. The spiral curves are partially stretched and drawn together with a certain rhythm, so that an actively motile organism resembles a spiral spring which is alternately drawn out and relaxed. When reduced in motility the organism may rotate along its axis in one and then in another direction without changing its curves. In certain species a lateral bending or swinging motion of one-half of the body may be seen. It seems to be the general rule that the more active and energetic an organism is, the less rigid are its curves. On the whole the pallidum group (Treponema) exhibits a less energetic motility than the heavier group (Spironema) which it relinquishes much sooner than the latter. Therefore, it is only in perfectly fresh material (such as that obtained from an experimental syphilitic lesion in animals at the moment of examination) that the stretching of the curves as in the case of so-called Spironema can be recognized. This point can be clearly demonstrated in a section of a syphiloma in a rabbit's testicle fixed immediately after removal from the animal. Here we find the organisms to show most striking irregularity of curves very unlike the accustomed picture of regularly curved specimens found in a section obtained from postmortem material such as a tissue from macerated congenitally syphilitic fœtus (Flexner [55]) or from a preparation made after the organism has become sluggish. The reverse is also true. A spironema from a case of relapsing fever is always wavy and irregularly curved in a stained preparation, but

it is much more regular when observed under the dark-field microscope, and becomes completely regular when nearing death as a result of being exposed to progressively unfavorable conditions. In a culture where the motility is somewhat less active the organism appears just as regularly curved as a treponema. The sudden death of these organisms leaves them in a state of motion, hence their irregular curves.

The cell-body of Spironema is much heavier than that of Treponema and in relation to different dyes it may be stated that the former takes on a more bluish component of Giemsa's solution than the latter, which usually takes on the red. In regard to the structure of the cell-body, the minuteness of these organisms precludes the possibility of obtaining much information by means of our present methods of differentiation. Many authors assume the presence of a membrane analogous to the periplast of a flagellate and believe that it can be demonstrated by means of maceration. In one species of Spironema, Prowazek [40] assumed a central axial filament surrounded by a layer of cytoplasm. The active motility exhibited by these organisms led some investigators to suggest the existence of contractile fibrils or a myoneme in the cell-body. My observations on fresh specimens obtained from pure cultures of these organisms support the view that the spironemata are provided with an axial spiral filament covered with a layer of protoplasm. On the surface of the cell-body there is a thin membrane which can be detected when the organism undergoes degeneration. At this stage the cytoplasm becomes so rarefied, *i.e.,* it escapes from the space which it occupied, that the axial filament and the membrane can be easily recognized. In a subsequent phase the membrane also disappears, leaving the axial filament denuded. This is a common phenomenon in the cultivation of this group of organisms. Schellack [19] maintains that the external layer of the cell-body stains red with iron hæmatoxylin eosin, while the inner layer takes on a dark bluish tint, hence the former is of ectoplasmatic and the latter of endoplasmatic origin. Gonder [56] describes an ectoplasmatic layer in Spironema vesperuginis. Fantham and Porter,[35] as well as Prowazek,[40] mention the existence in Spironemata of an undulat-

ing membrane, as was originally suggested by Schaudinn [56] owing to a wavy movement which he observed to travel through the body of a resting spironema. Gross and Zuelzer failed to demonstrate any such particular structure. Another important feature of Spironema and Treponema is the presence of a terminal appendage projecting from the end of the cell-body. The bodies of spironemata and treponemata taper at both extremities, from which is sent out a very fine terminal thread, at one or both ends. The length of the terminal appendage may reach ⅓ to ½ of the body and is immeasurably thin. In old cultures, especially when grown in a fluid medium, these terminal appendages are much heavier and more easily recognized than in a specimen derived direct from the natural habitat. The terminal filament is provided throughout its length with numerous, closely set, regular curves.[57] It is rigidly joined at the pointed ends of the body or sometimes in such a loose manner as to permit the joint to bend at any angle to the long axis of the organism. No proper motility can be discerned in the appendage, which is elastic. In certain specimens an active swinging or jerking movement can be seen to be transmitted by the organism, which is able to do this by means of its contractile element (myoneme?) contained within the body. In several instances in which the cultivated Spironema recurrentis had been exposed to the solvent action of certain chemicals (saponin, sodium taurocholate, etc.), I have observed many denuded axial filaments (their cytoplasmic layer having been dissolved) to which the terminal filaments were also attached. Suddenly I saw some of the terminal projections commence active jerking and swinging motions. The skeletal axial filaments still remained. By means of careful examination it was found that there were a pair of highly refractile, round bodies attached to the skeletal filaments near both extremities. These bodies, which measured about 0.5μ in diameter, appeared to have some contractility as suggested by the alternate change in the degree of the refraction of light. Whether or not these bodies represent some sort of myonematous elements cannot be definitely stated, but it is significant that similar nodules, if not in pairs, can be seen to travel from one point to

another in an actively motile spironema. Prowazek [58] once called attention to the phenomenon of plasmatic condensation in the body of Spironema gallinarum.

The nature of the terminal appendage is not known. Many authors (Hoffmann, Prowazek, etc., on S. buccalis and S. balanitidis; Novy and Knapp on S. recurrentis) view it as a prolongation of the periplastic fibrils which are in connection with the periplast. Others regard it simply as a drawn-out part of the cytoplasm produced at the line of division. I am inclined to think that the terminal projection with regularly set curves is a separate part not directly connected with the membrane, nor existing as a prolongation of the axial filament. It is connected with the cell extremity by means of a tendinous substance. It resembles the flagellum of certain bacteria, inasmuch as it is similarly elastic, finely set with regular curves, and visible under the dark-field microscope. On the other hand a great many of the bacterial flagella cannot be demonstrated in a fresh preparation even by means of a dark-field illumination. Zettnow, Borrel and Fraenkel [59] obtained preparations of S. recurrentis, S. gallinarum and S. duttoni in which peritrichal "flagella" were shown by means of flagella staining methods, but these flagella-like fibrils are now regarded as fibrils which have become detached from the external layer of the organisms through maceration. By means of the lucidol method of Szécsi,[60] Gonder [45] succeeded in staining one fine terminal projection at each end of S. recurrentis, as did also Wolbach by the adoption of Casares-Gil's [61] method.

There are several views regarding the mode of multiplication. The theory most generally accepted is that these spironemata undergo transverse division like bacteria, differing from the latter, however, in not forming a wall at the point of division. The division is effected by means of a thinning-out process of the protoplasma which for a time bridges the two newly-formed daughter cells. Finally they separate by the severance of the connecting thread. Novy and Knapp [18] described a cleft formation at the point of division. The view of the transverse division is held by Koch, Novy and Knapp, Metschnikoff, C. Fraenkel, Borrel, Laveran, Sobernheim, Gross, Thesing, Schellack, Nakano [62]

and others. On the other hand Schaudinn, Hoffmann, Hartmann, Keysselitz, Herxheimer, Prowazek, Gonder, Fantham and Porter support the theory of a longitudinal division as in the flagellates. Indeed, Krysztalowicz and Siedlecki [63] in 1905 went so far as to propose the term "Spiroflagellata" under Mastigophora. I have also observed instances in which the phenomena could only be explained by longitudinal division. Thus, in pure cultures of various spironemata and treponemata we find forms in which a longitudinal cleft can be traced in the somewhat heavier specimens. The cleft may run but a short distance or one-third, one-half or almost the entire length of the body. In some specimens the cleft widens up and causes one-half of the body to be split into two limbs (two daughter cells in half separation). Observed under the dark-field microscope the process is seen to be slow. It may be added that it is tedious to actually follow up the entire process of any mode of division under the microscope, no matter whether this be transverse or longitudinal. As may easily be conceived, those who held the theory of transverse division argue that the forms held by their opponents to be a stage of longitudinal division are formed by two entwined spironemata which, having been produced by transverse division, are still connected by a delicate plasmatic bridge. This argument, however, can also be used in the reverse sense in favor of longitudinal division, as it is also possible that the two daughter cells which have just undergone cell-division can remain united at their ends, thus bearing the appearance of representing a stage of transverse division. A strong support in favor of the transverse mode of multiplication lies in the formation of a very long thread consisting of several sections united by means of a delicate bridge between them. This phenomenon is of common occurrence in any spironema or treponema culture. It is highly probable that the usual mode of division in cultures is transverse, although the possibility of longitudinal division cannot be excluded. Recently Meirowsky [64] advanced the view that Spironema and Treponema besides multiplying transversely also do so by means of a process of fructification (Doldenbildung) and budding (Knospbildung) similar to that observed in some

lower plant organisms. His ideas were chiefly based upon phenomena observed by means of various methods of vital staining in a culture of Treponema pallidum (furnished by Sowade). He describes numerous granules collected in a group at one point or another along the body of the pallidum and also branching out of sprouts from some of the specimens. There are many factors to be taken into consideration in such an experimental arrangement which will make it difficult to properly estimate the value of the observations. Those made under the microscope on a preparation containing the organisms, consisting of semi-coagulated horse serum, solution of precipitable aniline dyes (effected particularly through a change of reaction in the medium) are of a disputable character when we consider the absence of strict aseptic precautions as well as the comparatively long period of observation (many days and weeks) during which a preparation had been kept for observation. It is possible that under these unfavorable conditions various forms of involution result which do not appear under normal cultural conditions. Certainly it is not convincing to admit that this so-called fructification or budding also occurs in the body of infected hosts.

Balfour [65, 66] noticed the appearance of certain granules within some of the erythrocytes of fowls which had just stood the first attack of the Sudanese fowl spironematosis and thought that these granules gave rise to a new generation of the spiral forms of the organism which reappear at the second attack. That is to say, that a Spironema found by Balfour in a Sudan epizootia possesses a spiral and a granular phase of life. Leishman [67] Blanc,[68] Fantham,[69] Nuttall [70] and Hindle [70] also entertain the belief that Spironema duttoni and Spironema gallinarum adopt a granular form under certain conditions, and that a spiral form can sprout out when the conditions become favorable. Thus, in the body of infected ticks these spiral organisms undergo segmentation, and numerous granules are produced, a process analogous to sporulation. These granules were called by the authors coccoid bodies, infective granules or spores. This view was supported by the histological studies of Hindle, who secured a series of

preparations in which these granules can be demonstrated in the body of the tick. According to Hindle these granules become spiral when the infected tick is incubated at 37° C. for a certain time. In contradistinction to the above findings Marchoux and Couvy,[71] Gleitmann,[72] Gonder,[45] Todd, and Wolbach[73] maintained that in an infected tick some motile spironemata can always be demonstrated and that the granules described by Hindle and others are not specific for the infected ticks, but can also be found in the control specimens. Fantham[69] points out, however, that the granules of normal ticks are not identical with the coccoid bodies of Spironema found in the infected ticks.

Schaudinn, Prowazek and others noticed that certain species formed nodules under adverse conditions and suggested that these may represent a resting stage (or resistant form); but Schellack and Wolbach regard them as a depression phenomenon which can also be induced by prolonged treatment of the organisms with a saline solution. Besides, there is a peculiar, highly refractile, round body which is very often found attached somewhere along the side of the body of the organism. There may be one or more such bodies in a specimen. The significance of this body is still obscure but it may possibly be caused through a disturbance of the osmotic equivalence existing between the cytoplasm of the organism and the medium, not unlike the phenomenon known as plasmaptysis. I have demonstrated its occurrence in the cultivated specimens of various species of Spironema and Treponema. The body is more frequently present in an old culture in which innumerable granules are also found. In certain culture tubes these minute granules are mostly of varying size. By making a transplant of such a culture into a new medium it was found that, when examined several days later, the new culture contained many short spiral forms which were in one manner or another intimately connected with the granules. This phenomenon suggested the possibility of representing the sprouting of the spiral forms from the granules.

Pathogenicity.—Spironemata and treponemata are parasitic, and some varieties are responsible for various diseases in man and

animals. Various forms of acute febrile diseases, as well as chronic pathological conditions, are caused by the invasion of the blood or tissues by this group of organisms. It may be mentioned that the spironemata are almost always transmitted from a sick individual to a normal person through the intermediary of certain blood-sucking insects and invade the blood principally, whereas the pathogenic treponemata are carried from man to man by direct contact and show a predilection for various organs and tissues. As a rule, the phase of the spironemal infection is acute and brief and that of the treponemal invasion runs a chronic course, as instanced in the former case by the type of relapsing and tick fevers and in the latter by syphilis and yaws.

Besides the pathogenic species there are a large number of saprophytic varieties belonging to these two genera (or one according to certain classifications) which are common inhabitants of the oral cavity, genitalia and alimentary tract of man and animals. Some forms are frequently associated with certain pathological conditions, but their etiologic significance has not been definitely determined. Such is the case with S. balanitidis in Ulcus erosiva circinata, S. vincenti in an acute angina, S. schaudinni in Ulcus tropicus and Treponema mucosum in pyorrhœa alveolaris, etc. It may be that some of these play the rôle of a secondary invader and aggravate the conditions.

In the following table I have enumerated the different species of Spironema and Treponema which have hitherto been observed by various investigators throughout the animal kingdom. It will be seen that the search has been more thorough in the case of the warm-blooded vertebrates than the cold-blooded orders, while even mosquitoes, ants, mites and fleas are found to harbor certain species of these organisms.

SPIROCHÆTÆ (LARGE FREE-LIVING FORMS)

S. plicatilis.*	Fresh water. 100–200μ, 500μ max. $\times$0.5–0.75μ	Ehrenberg, 1838
S. plicatilis marina *		Zuelzer, 1912.
S. plicatilis eurystrepta *		Zuelzer, 1912.
S. stenostrepta *		Zuelzer, 1912.
S. daxensis†	Hot spring. 30–100$\mu$$\times$0.5–0.75$\mu$	Cantacuzene, 1910.

* Zuelzer cultivated these varieties in a suitable medium and proved each of them to be different from the others; hence he made subspecies. Schaudinn considered them to represent male and female forms.

† In the water of hot springs of Dax (52°–56° C.).

CRISTISPIRÆ AND SAPROSPIRÆ (LARGE SAPROPHYTIC AND COMMENSAL FORMS IN THE ALIMENTARY CANALS OF SHELLFISH)

Cristispira balbianii‡	Ostrea angulata O. edulis	100–120$\mu$$\times$3–5$\mu$	Certes, 1882.
C. anodontæ	Fresh water mussel, A. cygnea; also A. mutabilis	130$\mu$$\times$3–4$\mu$	Keysselitz, 1906.
C. spiculifera§	" "	28–36$\mu$$\times$0.7–1.1$\mu$	Schellack, 1909.
C. primæ *	Prima squamosa, P. nobilis	10–60$\mu$$\times$0.5–3$\mu$	Gonder, 1908.
C. mactræ	Mactra sulcataria	45–70$\mu$$\times$0.8–1.0$\mu$	Prowazek, 1910.
C. pectinis	Pecten jacobæus	72μ $\times$1.5μ	Gross, 1910.
C. interrogationis		25μ $\times$0.5μ	Gross, 1910.
C. veneris	Venus casta		Dobell, 1910.
Saprospira grandis			Gross, 1912.
S. nana			Gross, 1912.

* Gonder once described a blepharoplast near one blunt end; nucleus in single rod or irregular masses. Specimens with rod-formed nucleus may be male elements, since they are highly active, the others female or indifferent elements. A concentration of all the chromatin into one rounded mass was sometimes observed. Encystment also occurs. Gonder no longer upholds his above-cited interpretations, explaining them on the ground of faulty technical handling of the preparation.

‡ Once considered to be trypanosome or spirochæta.

§ Bosanquet doubts its being a separate species from C. anodontæ.

	(Schellack)	Length Average	Length Extremes	Breadth Average	Breadth Extremes	Ends
C. balbianii	Ostrea edulis	39	35–42	1.3	1.1–1.5	Rounded, no t. ap.
C. ostræ	Ostrea edulis	41.5	38–42.5	1.1	1.0–1.3	Sharp, no t. ap.
C. chamæ	Chama gryphoides, Ch. sinistrorsa	45.6	45–46.5	1.4	1.3–1.5	Rounded, no t. ap.
C. anodontæ	Anodonta mutabilis	46	39–50.5	1.0	0.9–1.2	Rounded, no t. ap.
C. spiculifera	Anodonta	33	28–36.5	0.9	0.7–1.1	Pointed, t. filam.
C. modiolæ	M. barbato	37.5	36–40	0.8	0.7–0.9	Rounded, no t. ap.
C. primæ	P. nobilis	30.4	29–31	1.0	0.8–1.1	Rounded, no t. ap.
C. limæ	L. inflato, L. hiano	37	35–41	1.4	1.0–1.8	Rounded, no t. ap.
C. cardii papillose	C. papillosum	19.1	18.5–20	1.2	1.1–1.4	Rounded, no t. ap.
C. tapetos	T. decussata	34.5	29–35	1.3	1.1–1.4	Rounded, occasional t. ap.
C. acuminata	Tapes læta	37	43.5–49.5	1.0	0.9–1.1	Pointed, no t. ap.
C. saxicavæ	Sax. arctica	31	30–32	1.7	1.6–1.8	Rounded, no t. ap.
C. gastrochænæ	G. dubia	29	constant	1.2	1.1–1.3	One end blunt, one sharp, no t. ap.
S. pusella *	Anodonta, Umo, Lima, Tapes, etc.	13	12–14	...	0.3–0.4	Sharp pointed.

* Bosanquet found a spirochæta 10–12μ in length which he thinks may be identical with Spirochæta hartmanni of Gonder or with S. pusella of Schellack. No crista?

SPIRONEMA

S. obermeieri* ...Man, Europe. $8–16\mu \times 0.25\mu$ Colin, 1877 ([74]).
S. carteri........Man, India..$8–16\mu \times 0.2\mu$..Mackie, 1907 ([75]).
S. duttoni.......Man, West Africa..$16–30\mu$. $\times 0.2\mu$........Novy and Knapp, 1906, Breinl, 1906.
S. kochi.........Man, East Africa.........Schellack, 1907 ([77]).
S. berbera.......Man, Algiers........12μ..Sergent, 1908.
S. ægyptica......Man, Egypt ....13.5μ.
S. novyi†........Man, North America. 12μ..Schellack, 1907 ([77]).
S. ictero-hæmorrhagiæ.....Man, $4–9\mu \times 0.3\mu$, exceptionally 25μ.............Inada, 1914–15 ([79]).
S. nodosum......Man....................Hübener and Reiter, 1916 ([80]).
S. gallinarum‡ ...Fowl....................Marchoux and Salimbeni, 1903 ([81]).
S. anserina.......Goose ....Sacharoff, 1890 ([82]).
S. theileri.......Cattle. $20–30\mu \times 0.25–0.33\mu$. Laveran, 1902.
S. bovis caffris...Cattle..................Nuttall, 1910.
S. equi ....Horse...................Novy and Knapp.
S. equina........Horse...................Theiler, 1906 ([83]).
S. ovina..........Sheep...................Blanchard, 1906.
S. macaci........Inacacus, Ceylon..........Castellani and Chalmers, 1908.
S. pitheci........Cercopethicus pates........ French Sudan.............Thiroux and Dufongéré, 1910.
S. lutræ.........Otter....................Prowazek, 1907.
S. lovati........Grouse's coecum $16–32.5\mu \times$ Fantham, 1910.
S. vesperuginis...Tunisian bat. $12–18\mu \times 0.25\mu$ Gonder, 1908.
S. lagopodis.....Grouse's blood...$10–18\mu \times$ .Fantham, 1910.
S. laverani......Mouse. $1.8–3.75\mu \times 0.1–0.2\mu$. Breinl and Kinghorn, 1906 ([84]).
S. suis..........Pigskin lesion or tumor $6–12\mu$ Dodd, 1906, Cleland, 1906.
S. muris ....Rat...........$3–7\mu \times 0.2\mu$. Wenyon, 1906 ([85]).
S. minor........Rat............$5–9\mu$......Carter, 1887, ([86]).
S. microgyratum. Ulcerated cancers..$5–11\mu \times$ $1.5–2\mu$ $2.5–6\mu \times 0.16–0.25\mu$. Löwenthal, 1906 ([87]).

*Synonymous with S. recurrentis, Lebert, 1874.

† The designation of this variety as S. novyi originated in Schellack's article above quoted and it has since gained a wide acceptance. In going over the literature one cannot escape the impression that a better recognition ought to have been accorded Norris, who, with Flournoy and Pappenheimer, was the first to transmit this spirochæta from patients to ordinary laboratory animals and succeeded in securing a transient culture for two successive generations *in vitro*. Of course Novy deserves the credit of differentiating this variety from the closely allied types by immunity relations.

‡There are three subspecies: S. granulosa penetrano, in Sudan; S. Nicollii in Tunis, and S. neveuxi in Senegal.

S. eugyratum....Human intestine,
4.6–7.3μ..Werner, 1906.
S. stenogyratum .Human intestine.3.6–6.7μ .Werner, 1906.
S. gondii........Rodent Ctenodactylus gondi
16–19$\mu \times$0.3μ..........Nicolle, 1907.
S. gadi......S.W.Fish, Gadus minutus.......
10–16$\mu \times$3.5–4μ.......Neumann, 1909.
S. pelanychis S.W. Pelamys sarda....9–10$\mu \times$
1–1.9μ...............Neumann, 1909.
S. jonesi.F.W. ..Fish, Clavias angolensin....
18$\mu \times$0.1μ............Dutton, Todd and Toby, 1906.
S. hartmanni ....Prima squamosa,
P.nobilis intestine 6–14$\mu \times$1μGonder, 1908 ([88]).
S. bufonis ....Bufo vugaris Rectum
8–10$\mu \times$1.5μ..........Dobell, 1908.
S. minei.........Work ants. Termes lucifugus..15–50$\mu \times$0.3–1μ Prowazek, 1910.
S. glossinæ......Tse-tsefly stomach.8–15μ..Novy and Knapp, 1906.
S. culicis........Gnat. aliment. canal.large..Jaffé, 1907.
S. buccalis*...............12–20$\mu \times$0.5–xμ..Cohn, 1877.
S. vincenti......Pharyngitis.......10–40μ..Blanchard, 1906. ([89]) ([90]).
S. gracilis.......Abscess near jaw..........Vesprèmi, 1907 ([91]).
S. Schaudinni....Tropical ulcer............Prowazek, 1907 ([92]).
S. pseudopallidum Various ulcers............Mulzer, 1905 ([93]).
S. bronchialis....Bronchitis in Ceylon 15–30μ Castellani, 1907 ([94]).
S. phagedenis....Phagedenic ulcer in man...Noguchi, 1912 ([95]).
S. refringens................8–12$\mu \times$0.33μ..Schaudinn, 1905 ([6]).
S. balanitidis....Balanitis.................
8–12$\mu \times$0.5–0.75μ..Hoffmann and Prowazek, 1906 ([39]).
S. obtusum......Yaws lesion...............Castellani, 1905 ([96]).
S. acuminatum...Yaws...................Castellani, 1905 ([96]).
S. aboriginalis....Ulcerative granuloma on pudenda....18–20μ....Cleland, 1909 ([97]).
S. interrogans....Yellow fever...14$\mu \times$0.17μ.Stimson, 1909.
S. hyos..........Hog cholera..............King, Hoffmann, Bæslack, 1913 ([98]) ([99]) ([100]).
S. grassi.........Termite in Italy..........Doflein.
S. termitis ......Termite in Ceylon, large...Dobell, 1910.
S. ctenocephali...Dog flea..................Patton.

Lingard described Spironema in the blood of the camel, dog, elephant and horse; James, in an ulcer of the dog's muzzle, and Lucet in gastroenteritis; Mathis and Leger in the blood of the zebra and antelope; Bell and Ruquet in the stomach of a normal dog; Dobell in Tropidonatus stolatus:

*Subspecies: Undulata and inequalis.

Mühlens and Gleitmann in the boa constrictor; Ed. and St. Sergent in the alimentary tract of anopheles maculipenis larva; Mühlens often found spirochætes resembling S. recurrentis in the Culex mosquitoes.

TREPONEMA.

T. pallidum.....Syphilis .6–14μ×0.2–0.25μ.Schaudinn and Hoffmann, 1905 (6).
T. pertenue.....Yaws....................Castellani, 1905 (101).
T. microdentium .... 3–12μ×0.2–0.25μ..Noguchi, 1912 (102).
T. dentium* ....Koch, 1877.
T. macrodentium.........6–18μ×0.3–0.5μ..Noguchi, 1912 (102).
T. mucosum.....Pyorrhœa alveolaris.......
3–12μ×0.2–0.25μNoguchi, 1912 (103).
T. calligyrum....Condyloma...............Noguchi, 1912 (104).

The foregoing list may be classified according to the habitat of the organisms. Thus, when they multiply within the blood circulation of man or animals they may either lead to a grave pathological condition or may produce no appreciable disturbance of the host which harbors them. In the case of certain tissue-invading species, serious pathological consequences may ensue or the host may remain more or less indifferent to the parasite.

1. VARIETIES WHICH INVADE THE BLOOD PRINCIPALLY.

a. Those which cause characteristic fevers known as relapsing or tick fever (pathogenic). There are seven for man, five for mammals and at least two for birds. These are:

For Man.—Spironema obermeieri (in European relapsing fever), S. carteri (in East Indian relapsing fever), S. duttoni (African tick fever), S. kochi (in East African tick fever), S. novyi (in American relapsing fever), S. aegyptica and S. berbera (in Egyptian and North African relapsing fever).

For Mammals.—Spironema theileri (in South African cattle fever), S. equi and S. equina (in horse), S. ovinæ (in sheep), S. macaci and S. pitheci (both in East Indian monkeys).

For Birds.—Spironema gallinarum (in South American and African chicken fevers), S. anserina (in goose fever).

b. Those which do not seem to produce any grave condition, but are incidentally found (non-pathogenic).

For Man.—None.

*Subspecies: S. recta, S. tenue, S. denticola.

For Mammals.—Spironema lutræ (in otter), S. gondii, S. vesperuginus (Tunician bat), S. muris, S. minor (both in rats), S. laverani (in mouse). The organism found by MacNeal [105] may be identical with S. muris.

For Birds.—Spironema lagopidis (in grouse's blood).

For Reptiles.—Spironemata found in Tropidonotus and Boa.

For Fish.—Spiroonema gadi, S. pelamydis, S. jonesi.

2. VARIETIES WHICH INVADE THE TISSUE PRINCIPALLY.

a. Those which cause characteristic lesions and symptoms (pathogenic). In this group there are no Spironema, but the only two known varieties belong to Treponema. No pathogenic tissue parasite belonging to Spironemaceæ was found in animals. The two pathogenic treponemata for man are Treponema pallidum (in syphilis) and T. pertenue (in yaws).

b. Those which do not seem to cause any noticeable lesion. To this belongs a Spironema (or Treponema) discovered by Gaylord [106, 107] and Borrel [108] in mouse cancers. Similar organisms were found by Tyzzer,[109] Deetjen [110] and Mezinescu.[111]

3. VARIETIES WHICH INVADE BOTH THE BLOOD AND THE TISSUES INDIFFERENTLY.

Spironema (?) icterohæmorrhagiæ (in Weil's disease prevalent in Japan) and S. nodosum (in Weil's disease prevalent in Germany) is the only one so far known to come under this heading. This organism, first discovered by Inada, is probably identical with S. nodosum of Huebener and Reiter, who also found it independently of Inada a year later. Stokes confirmed the work of Inada on the cases prevalent in Flanders.*

4. VARIETIES WHICH MAY BE ASSOCIATED WITH CERTAIN PATHOLOGICAL CONDITIONS, AND SOME OF WHICH ARE REGARDED AS HAVING A MORE INTIMATE RELATION TO THE LESION THAN THAT OF MERE SECONDARY INVADERS.

There are about seven spironemata and one treponema which have been recorded in man and may be included in this category. These are: Spironema vincenti (acute pharyngitis), S. schaudinni (in tropical ulcers), S. bronchialis (in pulmonary gan-

* Personal communication from Dr. Adrian Stokes, Captain R. A. M. C.

grene), S. balanitidis (Ulcus erosiva circinata), S. gracilis (ulcerating jaw), S. aboriginalis (ulcerative granuloma), S. phagedenis (in phagedenic ulcer). The only treponema which may be constantly associated with pyorrhœa alveolaris is T. mucosum. S. suis was found in the ulcerating lesions of pigs.

5. VARIETIES WHICH ARE FOUND IN OR ABOUT THE BODY CAVITIES, ALIMENTARY TRACT AND GENITALIA, ARE NON-PATHOGENIC AND NUMEROUS.

For man there are described 8 species of Spironema and 5 species of Treponema. Some of them have been known for many years while others are of recent addition. It may here be noted that similar flora are encountered in mammalian animals. These are as follows:

Spironema refringens, S. microgyratum, S. buccalis, S. acuminatum, S. obtusum, S. pseudo-pallidum, S. eugyratum, S. stenogyratum, and Treponema macrodentium, T. medium, T. microdentium, T. dentium, T. calligyrum.

S. hyos, discovered by King and Baeslack [98] in the blood of pigs suffering from hog cholera, is considered by them to be the cause of this disease.[99, 100] This organism should be more extensively studied, particularly in its relation to various spirochætes found in certain conditions of this animal.[112,113]

The Spironema flora in birds, reptiles, fish and amphibia is practically unexplored, but we find one Spironema bufonis in a toad.

For insects there are on record several spironemata, namely S. culicis (culex mosquito), S. termitus, S. grassi (both in mites), S. ctenocephali (in dog fleas).

Before leaving this chapter it may be well to dwell somewhat more extensively on the two recently discovered pathogenic spironemata. Brief mention has been made of the one, namely Inada's S. icterohæmorrhagiæ, causing Weil's disease.[79] The other, discovered by Futaki, Takaki and Taniguoki [114] in the blood and glandular tissues in two cases of rat-bite fever in Japan, is a Spironema believed by them to be allied to S. recurrentis.

Since Weil [115] called attention to the existence of an infectious disease characterized by a sudden onset—chills, high fever, mus-

cular pains, jaundice, occasional hemorrhages in the skin, and acute nephritis—there have appeared numerous contributions to establish the entity of the disease. It has been found to break out among the soldiers in a barrack or among butchers or sewage-drainers. The mortality in European epidemics was rather low (about 15 per cent.). The disease reaches its maximum on the ninth or tenth day, and then gradually ends in a lysis which retards the recovery of health for about a month or longer. Death occurs before the end of the second week of the illness. A similar disease has been known in Japan for several years past. It was reported in certain districts (Kiushiu, Chiba, Shikoku) to have claimed many thousands of victims every year among the farmers, miners, and other laborers. Children under ten years of age are seldom affected, while those who are more occupied in field work contract the disease more frequently. There seems to be no authentic instance in which the disease was carried to another individual by direct contact. While many bacilli and cocci had been isolated and temporarily held to be the causative agent of this disease, no conclusive evidence had been adduced to support them. Jaeger [116] once described a *B. proteus fluorescens* as the cause of Weil's disease, prevalent in 1892.

Since 1912, Inada and his associates have undertaken an extensive experimental study of this disease and in 1914 succeeded in transmiting it to guinea-pigs. Macacus, rabbits, rats and mice were partially or completely refractory to the inoculation. The most important points of the work of these investigators are (1) the reproduction of the typical symptoms (fever, jaundice, acute nephritis, swelling, and fatty degeneration of the liver, generalized hemorrhages, subnormal temperature before death, etc.); (2) the fact that successful inoculation of the guinea-pig depends upon the period of the disease at which the blood was drawn from the patient, namely, no positive result was obtained with material taken after the first week of illness; and (3) the discovery of the Spirochæta, *S. icterohæmorrhagiæ* in the blood, visceral organs, glands, affected skin, and muscles, both in man and the experimentally infected guinea-pig.

It must be mentioned that the discovery of the Spironema was first made early in 1915 with the tissues and blood of the guinea-

pig, as the organisms are more abundant in experimental Weil's disease than in human cases. In October, 1915, an opportunity was afforded me to observe a number of cases of this disease occurring in China and, through the co-operation of Dr. Miyajima, some material for experimental studies was collected. One of the patients had had the attack a month previously and was at the convalescent stage. He was anæmic, thin, and moderately jaun diced. The urine (dark, turbid) was collected and inoculated into the peritoneal cavity of the guinea-pig. The animal started to show the typical symptoms (fever, jaundice, epistaxis, petechia, bile pigments in the urine, etc.) within one week and was examined just before death. The heart's blood showed *S. icterohæmorrhagiæ* in moderate numbers. They were motile (their curves were irregular and showed lateral twitching motions or some serpentine movements). Their length varied from 9μ to 12μ and the width was about 0.4μ. More organisms were seen in the emulsions of the liver and kidney. Some of the specimens were as long as 16μ and some as short as 4μ. The number of curves varied from 4 to 10. Inada, Ido, Hoki and others state that the body of the organism seems to be beaded when examined under the dark-field microscope. Like other minute treponemata or spironemata, the unstained Spironema of Weil's disease is invisible under the ordinary microscope. When stained with the Giemsa, carbol fuchsin, gentian violet, or Fontana stains, the organism presents a spiral thread possessing only a few large curves with pointed extremities. There is a certain resemblance to Vincent's spirochæta, although it is somewhat smaller and finer than the latter. A flagellum has not been demonstrated, but in a preparation stained according to the modified Fontana method,* I was able to see a delicate projection drawn out of the pointed end of the organism. Probably there is a terminal thread. It is quite astonishing, however, to find that the organisms stained

* Fix air dried film in (1) a mixture of acetic acid 8 cc., formalin 20 c.c. and distilled water 100 c.c., for a few minutes; rinse off the reagent. Cover the film with a 2a mixture of 20 per cent. tannin plus 1 per cent. phenol and steam it over a flame for one minute; wash the film and then immerse the slide in a 0.25 per cent. silver nitrate solution for a minute or two. After washing cover the film once more with (2) and steam it over a flame; wash and dry.

by the Levaditi method appear to be very heavy, irregular forms with a few tortuous bands and blunt ends. By applying a modified technic [117] the organisms stain much more elegantly and preserve their delicate appearance.

As will be mentioned later, the *Spironema icterohæmorrhagiæ* has been successfully grown on artificial media and the disease reproduced in the guinea-pig by means of the pure culture.

Huebener and Reiter [80] reported early this year (1916) that they were also able to find a spirochæta in the experimental Weil's disease in the guinea-pig. The spirochæta, designated *S. nodosa* by them, seems to be identical with the strains isolated by the Japanese investigators. As briefly referred to, Stokes has just isolated the same organism for the Weil's disease existing in Belgium. He also succeeded in reproducing the typical disease in guinea-pigs in which the organisms were demonstrated in abundance.

The report of Futaki and his associates on the finding of a spironema in the inflamed skin and lymph-glands in two cases of rat-bite fever * is interesting, inasmuch as the clinical feature of this disease had already suggested to Crohn [118] its possible relation to recurrent fever. Hata and others had found an effective therapeutic agent in salvarsan and mercury. These spironemata were found to be actively motile when examined by the dark-field microscope, and were successfully transmitted to the Inus monkey, guinea-pig, and white rat for many generations. The organism discovered by Futaki appears to be allied to the blood spironemata of relapsing fevers. In the meanwhile this will raise an interesting question in regard to the possible existence of a spontaneous spironema infection in rats. So far as I am aware, there is no observation on record of the discovery of any pathogenic Spironema in the rat, notwithstanding the fact that this animal had been much hunted up and examined by health officers for the plague bacilli, thus affording numerous opportunities to make an

* Symptoms: Incubation of 10–27 days, then chills, fever, headache and malaise. Local inflammation at the site of bite: pains in the limbs of the affected side, dark red eruptions and swollen lymph-glands; 3–7 days' fever with an afebrile interval of 2–3 days. Temperature curve similar to that of relapsing fever.

accidental discovery. Perhaps the finding of Futaki may open up a new field wherein to search for a hitherto undiscovered source of disease communicable to man.

Viability.—There are a great deal of experimental data bearing upon the viability of various spiral organisms generally and especially upon the most widely investigated species *Treponema pallidum.* In recording the results it is necessary to make a distinction between experiments made with uncultivated organisms and with those which have already adapted themselves to the artificial cultural conditions, in view of the fact that the latter offer a much greater resistance to certain external influences.

The free-living spirochæta lives for about a week or ten days when taken out of its natural habitat and placed in a vessel without the observance of any special precautions. On the other hand, Zuelzer [22] was able to keep various free-living species of Spirochæta (plicatilis type) alive for an indefinite period of time by keeping them in a hermetically sealed vessel in which a sufficient amount of hydrogen sulphide and certain organic matters derived from stagnant water were supplied from time to time; in other words, in a culture.

The maximum time during which Cristispira can be kept alive is about two days even under favorable conditions. No culture has yet been obtained with any member of the shellfish parasites.

For Spironema it was found that the pathogenic varieties, including *S. recurrentis, S. duttoni, S. novyi, S. gallinarum,* still remain infective after a little more than 40 days when kept in a refrigerator (2°–4° C.).[18] At body temperature (37° C.) complete disintegration of the organism takes place within 48 hours. No accurate data can be found regarding the saprophytic species which, it may be assumed, can remain alive much longer than their pathogenic congeners.

Of the Treponema group, *Treponema pallidum* has received most attention. Authors agree that the syphilis organism quickly becomes sluggish after being removed from the living tissues and that motility can seldom be detected in any specimen which has been maintained at 37° C. for 24 hours. On the other hand, the pallidum contained in a resected tissue (for example, a piece of chancre or rabbit's testicular syphiloma) is still found to be

infective after being kept at room temperature or in a refrigerator for 48 hours or sometimes even 72 hours.* In a culture medium consisting of rabbit's plasma, a piece of rabbit's kidney and ascitic fluid, many pallida introduced in the form of an emulsion of rabbit's testicular syphiloma remain quite active for 3 or 4 days when kept at 37° C. under anærobic conditions. But they do not always multiply to form a real culture. It was found that post-mortem material containing *Treponema pallidum* may still be able to infect a susceptible animal when inoculated within 24 hours.[119] The organism is killed at a temperature between 50° and 55° C. maintained for thirty minutes.

The resistance and viability of cultivated strains of *T. pallidum* are much greater than those of the organisms found in the tissues. Akatsu, working in my laboratory, found that when the pallidum is isolated from a fluid culture and put in a fraction of a c.c. of the same fluid, it invariably dies within 24 hours, no matter whether it be kept at 37°, 15° or 2° C., but it survives for 5 days at 37°; 7 days at 15°; and 10 days at 2° C. when kept in 2 c.c. of the fluid. On the other hand, a small portion of a solid culture set aside in a tube remains capable of transplantation into a new medium for 48 to 72 hours at 15° C. and for 4–5 days at 2° C. In a quantity of about 2 c.c. of the culture the organism remains alive as long as twenty days.

In undisturbed cultures *T. pallidum* remains alive for a considerable length of time. Thus a solid culture, set up according to the original method,[120] will remain transplantable to a new medium for a period of one year uninterruptedly kept at 37° C. At 15° C. it remains alive after standing 4 or 5 months, while in a refrigerator (2° C.) it survives about 2 months. In a fluid medium consisting of ascitic fluid and a piece of fresh rabbit's kidney covered with fluid paraffin, the organism lives about 2 to 3 months and in a double tube method [121] about 4 months at 37° C.

T. calligyrum, T. mucosum, T. microdentium are about the same as the pallidum in regard to their resistance and viability. These organisms resist the action of the sun's rays when exposed directly for several hours (4 hours and 30 minutes) at a tempera-

*Isolated specimens die within 10 hours in a refrigerator (Neisser).

	Treponema Pallidum	Spironema Recurrentis	Cristispira Anodontæ	Spirochæta Plicatilis
Saponin............	10 per cent. solution: 30 min., immobilized, irregular, paler. 1 hour: mostly broken up. Kills in 1: 75,000 dilution	Like pallidum when treated in 10 per cent. solution	10 per cent. solution: 1–2 hours, crista fibrillar, and then indistinct	10 per cent. solution: still motile in 30 min.; longer contact makes the body shadowy, but no dissolution.
Sodium taurocholate..	10 per cent. solution: like the above; kills in 1:2,500 dilution	10 per cent. solution: immobile in 15 min. The outer layer shrinks into irregular masses, exposing axial filament. Final disintegration	10 per cent. solution: destroyed in 15 minutes	Same as Saponin.
Sodium glycocholate..	Same as sodium taurocholate			
Sodium cholate.......	Same as above, but kills in 1:5,000 dilution.			
Sodium oleate........	10 per cent. solution: Same as above, kills in 1:70,000 dilution	Almost dissolved in 1 hour, but some may still be motile.		
Cobra lecithid........	Kills in 1:1,000 dilution.			
Cobra venom........	Kills in 1:1,000 dilution.			
Pepsin (0.1 in 150 c.c. of 0.3 per cent. HCl)	Cells swell up in 2 hours	....	Slight change.....	Granules appear in 2 or 3 days at 40° C., but only slight change at lower temperature.

Trypsin (0.2 in 10 c.c. of 0.5 per cent. Na_2CO_3)	Resist the tryptic digestion for many days	....	Crista, chambers and contents disappear in 24–48 hours. Membrane resistant	Granules and axial filament made distinct in short contact. At 40° C., for 2 or 3 days, only the axial filament remains. It may break into many pieces corresponding to curves.
H_2SO_4..............	1 per cent. solution: Immobilized immediately, shortened, granular, swollen, indistinct curves. 1 hour the same. 30 per cent. solution: dissolves the organisms	1 per cent. solution: complete immobilization; many appear thinner, but forms well-preserved. 30 per cent. solution: dissolution	30 per cent. solution dissolves them	1 per cent. solution causes immediate stretching of curves, which resume their winding when adding 1 per cent. KOH, or vice versa. This can be repeated many times. 30 per cent. solution dissolves the spirochæta.
KOH..............	10 per cent. solution: rendered indistinct in 30 min.; dissolution in 1 hour	10 per cent. solution: dissolves most of them in 1 hour; more resistant than the pallidum	1 per cent. solution destroys Crista; membrane resists 10 per cent., but dissolved in 30 per cent. with heat	1 per cent. solution dissolves granules, 2–30 per cent. destroy spirochæta, axial filament most resistant. Treatment with absolute alcohol accelerates the dissolving power of KOH.
Na_2CO_3............	1 per cent. solution: immobilized, but no morphological changes	1 per cent. solution: immobilized, slightly granular, but well preserved	....	1 per cent. solution: no effect on plasma, dissolves granules.

ture of 30° C. (summer) and 4° C. (winter), although no growth was obtainable with material exposed for 12 hours (Akatsu).

Drying promptly kills them, that is, no growth can be obtained by transplanting the dried cultures into new media.

The thermal death points for *T. pallidum* as tested out with pure cultures are as follows:

	5 min.	10 min.	15 min.	30 min.	60 min.
45° C.	+	+	+	+	+
50° C.	+	+	+	+	+
55° C.	+	+	—	—	—
60° C.	—	—	—	—	—
65° C.	—	—	—	—	—

The above data were obtained by Akatsu and closely agree with those obtained by Bronfenbrenner,[122] who found that the several strains of *T. pallidum* were destroyed at slightly lower temperatures. It must be stated that Bronfenbrenner used isolated organisms suspended in saline or ascitic fluid, while Akatsu subjected them to the action of heat in a thin culture tube.

Microchemical Reaction.—As mentioned elsewhere, a number of substances have been found to exert a dissolving or disintegrating action upon so-called " spirochætes " in general as well as upon certain protozoa. This phenomenon is claimed by certain authors to be decisive enough to place the spirochætes among protozoan organisms as the majority of bacteria (pneumococcus is an exception) remain unaffected, and some can multiply freely in a saponin solution which destroys spirochætes. While a too far-reaching generalization from these observations may be avoided, these reagents nevertheless furnish us with an excellent means of studying the microchemical structure of the organisms.

The preceding table contains a summary of all available data which however are very fragmentary and incomplete.

As will be noticed in the table, certain reagents demonstrate the existence of a resistant membrane in Cristispira, a trypsin resistant axial filament in Spirochæta, and a shadowy sheath (?)

as well as an axial spiral filament in Spironema and Treponema. As in the case of Spirochæta no true dissolution of Spironema (both gallinarum and recurrentis) or Treponema was effected by the saponin, but after several hours' contact they were shrivelled and broken up into irregular pieces.

Resistance to Disinfectant and Chemotherapeutic Agents.—Attempts to determine the resistance of various "spirochætes" are not lacking, but no satisfactory and accurate results were to be expected from the experiments in which their death point had to be determined through the intermediary of susceptible animals. Since the successful cultivation of different "spirochætes" has been effected, it has become possible to determine the effect of different chemicals. The following table shows a summary of the results obtained in two independent series of experiments by the use of common disinfectants.

RESISTANCE TO CHEMICALS

At 37° C.	
Lugol	kills in 1:3 dil.; 1:5–1:10 in 15 min.; 1:50 not in 1 hour.
Bichloride of mercury	kills in 1:5000 dil.; 1:10,000 in 15 min.; 1:50,000 in 30 min.; 1:100,000 not in 1 hour.
At Room Temperature.	
Phenol	kills in 1:200; 1:1000 in 30 min.; 1:5000 not in 1 hour.
Lysol	kills in 1:1000; 1:5000 not in 1 hour.
Formalin	kills in 1:200; 1:500 in 15 min.; 1:1000 not in 1 hour.
Potassium permanganate	kills in 1:1000; 1:5000 in 15 min.; 1:10,000 not in 1 hour.

Turning our attention to the chemotherapeutic agents it is scarcely necessary to remark that, thanks to the pioneer work of Ehrlich and his collaborators, especially to his contribution to our chemical treatment of spironematoses and trypanosomiasis,

a new field of scientific research has been inaugurated. Thus Morgenroth initiated a chemotherapy for bacterial diseases by discovering various quinin derivatives as a specific for pneumococcus. Flexner and Clark, the collaborators of Jacobs and Heidelberger,[123] made an extensive series of experiments in order to discover an effective chemical compound to combat poliomyelitis, wherein they obtained some encouraging results. In their early work they had employed numerous new derivatives of urotropin (hexamethylenetetramine) as this substance was known to penetrate into the intrathecal space. The work has since been extended to include various bacterial infections as well [124, 125, 126, 127] as trypanosomiasis and spironematosis (Brown and Pearce) with the use of additional new arsenic and mercurial compounds. While I do not wish to assert that the therapeutic effect of a chemical compound has any direct relation to the latter's disinfecting or sterilizing power against the causative agent *in vitro,* it was nevertheless thought of interest to find out how these new compounds, including various derivatives of urotropin, arsenic and mercury, would behave in relation to the various species of Spironema and Treponema in cultures.

It is a well-known fact that atoxyl arsacetin or arsenophenol, or even salvarsan, attack the trypanosomes and spironemata only after being introduced into the body, where they undergo reduction and produce a highly parasitotropic component. Yet, as will be shown in the following table, salvarsan is by no means inactive *in vitro* against *T. pallidum*. It is a fairly powerful treponemicide. Hence it is not without interest to study these compounds *in vitro* and then, when completed, compare the results with their therapeutic effects *in vivo.* The test tube determination of the germicidal property of these substances should form a part of our knowledge in perfecting chemotherapy. With the co-operation of Dr. Jacobs, who is in charge of the preparation of chemotherapeutic agents at the Rockefeller Institute, the following compounds were tested on cultivated strains of *T. pallidum in vitro* with the results indicated in the tables. A fuller report will be made later by Dr. Akatsu.

Table I gives a general survey of these compounds, while

TABLE I

No.	Preparation	Concentration sufficient to kill T. pallidum	Concentration which no longer kills T. pallidum
9	*p*-Bromobenzylhex. chloride	1 : 1,000	1 : 2,500
16	*o*-Xylylenedi-hex. chloride	1 : 2,500	1 : 5,000
19	2-Nitro-3,4-Dimethoxybenzylhex.chloride	1 : 2,500	1 : 5,000
21	1-(ω-chlorobenzyl)-2-oxy-3-naphthoic methyl ester)+hex	1 : 2,500	1 : 5,000
28	5-Chloromethylvanillin+hex	1 : 750	1 : 1,000
29	5-Chloromethylsalicylic acid+hex	1 : 2,500	1 : 1,500
40	*p*-iodobenzylbromide+hex	1 : 750	1 : 1,000
46	*o*-nitrobenzylchloride+hex	1 : 250	1 : 500
47	*p*-nitrobenzylhex. chloride	1 : 750	1 : 1,000
50	Methylhex. iodide	1 : 100	1 : 250
84	Chloroacetamide+hex	1 : 1,000	1 : 2,500
86	Oxymethylchloroacetamide+hex	1 : 250	1 : 500
90a	Ethyl bromoacetate+hex	1 : 1,000	1 : 2,500
96	Chloroacetylaniline+hex	1 : 1,000	1 : 2,500
97	β-acetoxy-α-chloroacetylnaphthobenzylamine+hex	1 : 1,000	1 : 2,500
102	Chloroacetyl-α-naphthylamine+hex	1 : 500	1 : 750
107	Chloroacetylbenzylamine+hex	1 : 500	1 : 750
109	Chloroacetyl-β-naphthylamine+hex	1 : 1,000	1 : 2,500
111	*o*-Methylchloroacetylbenzylamine+hex	1 : 2,500	1 : 5,000
112	Chloroacetyl-*p*-aminobenzoic ethyl ester +hex	1 : 1,000	1 : 2,500
114	Chloroacetylurea+hex	1 : 1,000	1 : 2,500
121	Phenoxyethylhex. bromide	1 : 250	1 : 500
122	*p*-Bromochloroacetylaniline+hex	1 : 2,500	1 : 5,000
126	Chloroacetylaminoazotoluene+hex	1 : 250	1 : 500
134	Chloroacetyl-*p*-anisidine+hex	1 : 2,500	1 : 5,000
138	Chloroacetylphenylhydrazine+hex	1 : 750	1 : 1,000
142	Chloroacetothylamide+hex	1 : 1,000	1 : 2,500
146	Menthyl bromoacetate+hex	1 : 750	1 : 1,000
147	Bromoethylphthalimide+hex	1 : 1,000	1 : 2,500
148	*p*-nitrobenzoic bromoethyl ester+hex	1 : 250	1 : 500
150	Bromoethyl benzoate+hex	1 : 500	1 : 750
158	β-Iodopropionyl-*o*-anisidine+hex	1 : 1,000	1 : 5,000
163	*p*-ethoxyphenyl bromomethyl ketone+hex.	1 : 500	1 : 750
164	Chloroacetyl-ψ-cumidine+hex	1 : 2,500	1 : 5,000
168	*p*-Acetamino-ω-bromoacetophenone+hex.	1 : 750	1 : 1,000

No.	Preparation	Concentration sufficient to kill T. pallidum	Concentration which no longer kills T. pallidum
171	*m*–Chloroacetylaminomethylbenzamide + hex.	1 : 1,000	1 : 2,500
172	*m*–Chloroacetyl–α, α,–phenylbenzylhydrazine + hex.	1 : 2,500	1 : 5,000
174	Chloroacetyl–aminoethyl anisate + hex.	1 : 500	1 : 750
204	3–(ω Bromoacetyl) quinaldine + hex.	1 : 2,500	1 : 5,000
218	Tribromo–*p*–cresyl bromoethyl ether + hex.	1 : 2,500	1 : 5,000
219	Chloroacetyl–*p*–aminoleucomalachite green + hex*	1 : 5,000	1 : 7,500
229	Chloroacetyl–*p*–aminobenzeneazo–p′–dimethylaniline + hex.*	1 : 500	1 : 750
232	*p*– Chloroacetylaminobenzeneazo – p′ – diethylaniline + hex.	1 : 1,000	1 : 2,500
234	α–naphthyl bromoethyl ether + hex.	1 : 500	1 : 750
239	*o*–Acetaminophenyl bromoethyl ether + hex.	1 : 1,000	1 : 2,500
242	*p*–chloroacetylaminodiethylaniline + hex.	1 : 1,000	1 : 2,500
244	Hex. + chloroacetylaminoethyl *p*–nitrobenzoate	1 : 2,500	1 : 5,000
249	Chloroacetyl–*p*–aminodipropylaniline + hex.*	1 : 500	1 : 750
252	Chloroacetyl – *p* – aminotetraethyl – p′, p″,-diaminotriphenylmethane + hex.	1 : 1,000	1 : 2,500
253	Chloroacetyldiethylamine + hex.	1 : 1,000	1 : 2,500
255	*p*–Cyanobenzylhex. chloride	1 : 1,000	1 : 2,500
257	Chloroacetyl–*o*–aminophenyl benzoate + hex.	1 : 1,000	1 : 2,500
261	Chloroacetyltriphenylmethylamine + hex.	1 : 1,000	1 : 2,500
262	Chloroacetylleucoauramine + hex. (* ?)	1 : 1,000	1 : 2,500
263	Chloroacetylaminoethyl *o*–nitrobenzoate + hex.	1 : 1,000	1 : 2,500
267	Chloroacetylaminoethyl β–naphthoate + hex.	1 : 2,500	1 : 5,000
271	Chloroacetyl – N – phenylaminoethyl – *p* – nitrobenzoate + hex.	1 : 1,000	1 : 2,500
272	*m*–Acetamino–*p*–tolyl ω–iodoethyl ketone + hex.	1 : 5,000	1 : 7,500
273	Chloroacetylethylaminoethyl *p*–nitrobenzoate + hex.	1 : 1,000	1 : 2,500

No.	Preparation	Concentration sufficient to kill T. pallidum	Concentration which no longer kills T. pallidum
278	α, β-Diphenylchloroacetylamino-ethanol +hex	1 : 250	1 : 500
280	Chloroacetyl-*m*-aminoacetophenone+hex	1 : 1,000	1 : 2,500
282	α-Phenyl-α-oxy-β-chloroacetylamino-ethane+hex	1 : 1,000	1 : 2,500
283	*p*-nitrobenzoylaminoisopropyl chloroacetate+hex	1 : 500	1 : 750
288	Iodopropanol+hex	1 : 500	1 : 750
289	2-Chloroacetylamino-3-oxy-3-methylbutane+hex	1 : 2,500	1 : 5,000
291	Chloroacetyl-*o*-methylphenoxyethylamine +hex	1 : 1,000	1 : 2,500
293	Chloroacetyl-β-amino-γ-butanol+hex	1 : 250	1 : 500
298	β-Phenyl-β-oxy-γ-chloroacetylaminopropane+hex	1 : 1,000	1 : 2,500
301	β-Naphthyl bromethyl ether+hex	1 : 1,000	1 : 2,500
303	2-oxy-3, 5-dibromobenzyl bromide (+?) +hex	1 : 1,000	1 : 2,500+
308	Chloroacetyl-*m*-iodoaniline+hex	1 : 750	1 : 1,000
309	Chloroacetyl-5-iodo-*o*-toluidine+hex	1 : 750	1 : 1,000
M1	(4-[p-oxybenzeneazo]-phenylmercuric acetate)	1 : 50,000	1 : 75,000
M4	[o-oxybenzylideneamino] phenylmercuric acetate†	1 : 50,000	1 : 75,000
M7	1-Amino-2-[*p*-naphthaleneazophenylmercuric acetate]-5-sulfonic acid	1 : 25,000	1 : 50,000

Hex. = hexamethylenetetramine.

* = Grind up in a mortar with a little water and add $\frac{N}{10}$ HCl carefully until dissolved.

† = Treat as above, using $\frac{N}{10}$ NaOH instead of HCl.

Table II puts down the strengths of various well-known disinfectants and chemicals for the sake of comparison. Table III gives the resistance of several culture strains of pallidum and other allied species to the action of two different new compounds.

As briefly mentioned, the spironemicidal (or treponemicidal)

TABLE II

Names of substances	Concentration sufficient to kill T. pallidum	Concentration in which T. pallidum survived
Phenol	1 : 2,500	1 : 5,000
Formalin	1 : 750	1 : 1,000
Lysol	1 : 5,000	1 : 7,500
Sublimate	1 : 100,000	1 : 500,000
Salvarsan	1 : 7,500	1 : 10,000
Neosalvarsan	1 : 2,500	1 : 5,000
Atoxyl	1 : 10	1 : 25
Sodium iodide	1 : 10	1 : 25
Potassium iodide	1 : 10	1 : 25
Lugol's solution	1 : 75	1 : 100
Iodox. benz. acid	1 : 500	1 : 1,000
Trypozofrol	1 : 25,000	1 : 50,000
Nectrypozofrol	1 : 250	1 : 1,000
Sodium cholate	1 : 5,000	1 : 7,500
Sodium glycocholate	1 : 2,500	1 : 5,000
Sodium taurocholate	1 : 2,500	1 : 5,000
Sodium oleinicum	1 : 25,000	1 : 50,000
Saponin	1 : 75,000	1 : 100,000
Cholesterin	No action	No action
Cobra lecithid	1 : 1,000	1 : 5,000
Cobra venom	1 : 1,000	1 : 5,000

TABLE III

Names of organisms	Preparation M1			Preparation No. 253	
	$\frac{1}{10,000}$	$\frac{1}{25,000}$	$\frac{1}{50,000}$	$\frac{1}{1,000}$	$\frac{1}{2,500}$
T. pallidum, heavy type	..	−	+	−	+
T. pallidum, thin type	−	+	..	−	+
T. calligyrum	..	−	+	−	+
T. mucosum	..	−	+	−	+
T. microdentium	..	−	+	−	+
S. refringens	..	−	+	−	+

power of salvarsan and neosalvarsan is alleged to increase considerably when introduced into the living body. In a series of experiments,[122] it was found that by allowing a sterile extract of freshly removed rabbit's liver or defibrinated blood of the same animal to act upon neosalvarsan for three hours at 37° C. the germicidal power of this drug increased from 1:1000 to 1:2000 in the case of the liver extract, and from 1:1000 to 1:5000 in the case of the blood. The addition of boiled extract had no such activating effect.

Acquisition of Increased Resistance to Drugs.—It will be recalled here that the failure of chemotherapy of trypanosomiasis in man and animals is partly due to the production of so-called drug-fast strains of various trypanosomes after the latter have on several occasions been subjected to the action of certain arsenic compounds. These organisms will be destroyed to a great extent by the first injection of the drugs, but if there remain a few which have resisted the first medication, they will multiply and the animal will once more be infested with the organisms. The offspring is more resistant to the action of the same drug than the preceding generation. A large dose of the medicament is necessary to destroy the organisms and to overcome this increased resistance. But as a matter of fact, the increased resistance of the organism to the drug is relatively much greater than that of the infected hosts, and the limit will soon be reached beyond which the quantity of the drug cannot be further increased without seriously affecting the infected man or animals.

Experiments of this nature have been made with atoxyl, arsacetin, arsenophenylglycin, etc. To employ Ehrlich's terms, the organotropic affinities of these drugs were so close to the parasitotropic, that it was impossible to employ a sufficient quantity to completely sterilize the infected body, since the administration of such a quantity would mean death or the serious impairment of some of the functions. Ehrlich's conception of a specific chemotherapy was based upon the fact that different cell groups are provided with their characteristic receptor apparatus (chemoceptor), to which a given chemical molecule attaches by means of its side chains. Thus, for trypanosomes there are certain receptors which will fit in with a certain atom complex of

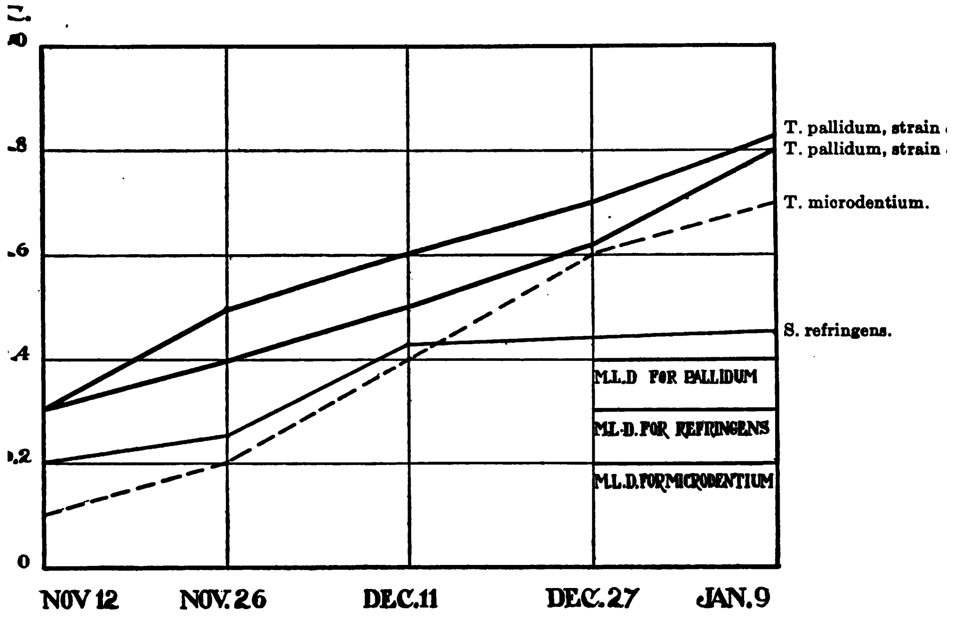
atoxyl, arsacetin, etc., while of the infected hosts the organs show much less affinity for them. In developing chemotherapy for syphilis, Ehrlich finally evolved a compound in which the spironematropic atom complexes were far more in excess than the organotropic groups. This compound, as is universally known, is dioxydiamidoarsenobenzol, better known as salvarsan. According to Hata [128] the ratio of the dosis curativa and dosis tolerata of this compound is 1: 3 for mice and rats infected with *Spironema recurrentis,* and 1: 58 for chickens with *S. gallinarum,* while in the case of experimental chancre in rabbits it is between $^{1}/_{7}$–$^{1}/_{10}$. In these animals Ehrlich's *Therapie sterilisans magna* was achieved, as also in cases of relapsing fevers in man. In human syphilis, however, in spite of the most powerful spironemicidal action, his original aim to sterilize the syphilitic body with a single injection of a large dose was not uniformly attained.

Yet there is no doubt that a prompt administration of salvarsan in sufficient dose during the early stage of infection sterilized the patients, as was evidenced by the increased instances of permanent abortion of the infection and of reinfection after the salvarsan treatment. On the other hand, we are also confronted with repeated recidives in certain patients. We often hear of mercury resistant as well as salvarsan refractory cases. It has been known for some time that *Spironema recurrentis* as well as *Spironema duttoni* produces an arsenic-fast strain in mice or rats when the latter are treated with atoxyl, arsacetin, etc. In this respect these spironemata resemble trypanosomes. Marks [129] once considerably raised the resistance of a bacteria to arsenious acid by allowing it to accustom itself gradually to the action of this chemical in test tube cultures. It therefore seems not at all improbable that Spironema as well as Treponema become more resistant to the parasitotropic effect of arsenic compounds and possibly of mercurial salts, not only *in vivo,* but *in vitro.* Akatsu [282] carried out a number of experiments in my laboratory in which he has apparently succeeded in raising to many times their original degree the resistance of the Treponema group to salvarsan, neosalvarsan, and bichloride of mercury. The experiments were carried out with cultures of these organisms, the

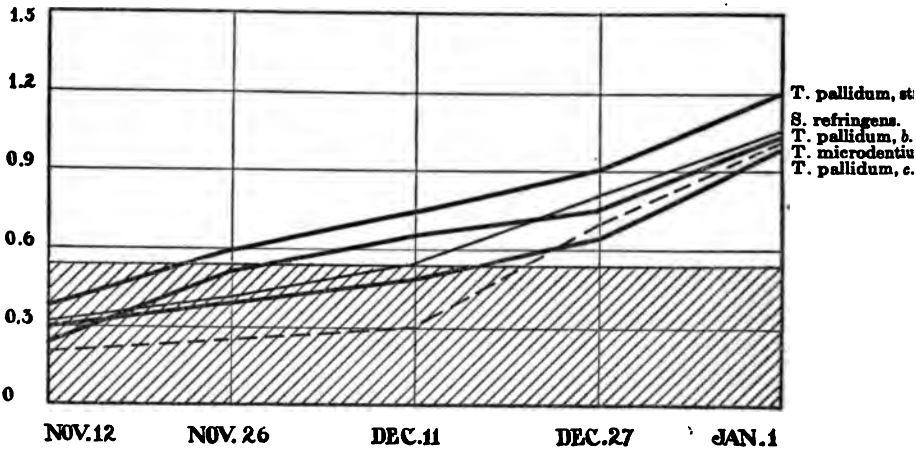
general plan being to cultivate the organisms in media containing these substances in a concentration just short of that required to suppress the growth completely, and to make subcultures from it into new media containing somewhat greater quantities of the chemicals than the preceding series. In the present experiments fluid cultures consisting of ascitic fluid and a piece of fresh rabbit's kidney covered with a layer of liquid paraffin were employed. Subcultures from one medicated culture to another were made at two weeks' intervals, during which time the general condition of the cultures could be estimated. As mentioned above, subcultures are made from tubes still showing numerous actively motile organisms. It is difficult to carry on the culture if one attempts to make a subculture in which too much medicament is present to give a fairly good growth, since no growth will be obtained in a subculture which has been inoculated with a poor culture arrested in its development by an excess of the drugs.

The results of our experiments may be summarized in the following charts:

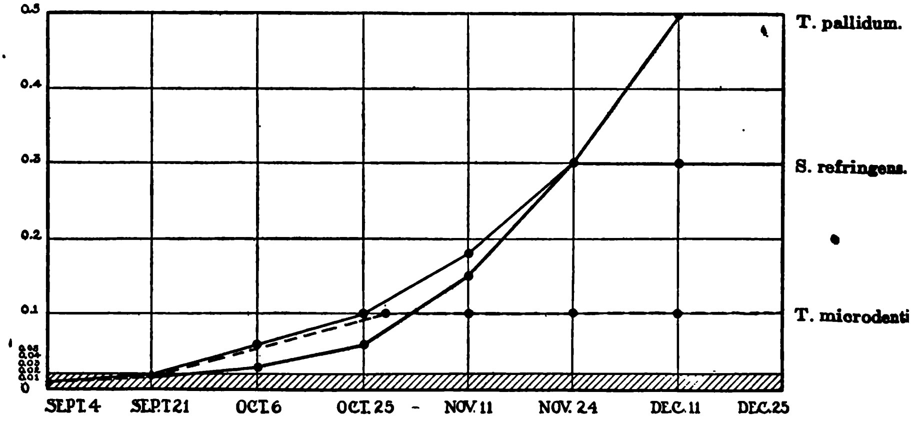
1: 10,000 dilution of Salvarsan in 5 c.c. of medium.



1: 10,000 dilution of Neosalvarsan in 5 c.c. of medium.



1: 1000 dilution of Bichloride of Mercury in 5 c.c. of medium.



In order to suppress the growth of various treponemata which had not previously been in contact with these compounds, the following doses were found necessary in a total volume of 5 c.c. of the culture medium. The solutions of salvarsan and neosalvarsan were 1: 10,000 dilution in water, and bichloride of mercury in

1:1000 dilution. Salvarsan was neutralized with NaOH, as usual.

	Salvarsan	Neosalvarsan	$HgCl_2$
Refringens	0.4 c.c.	0.6 c.c.	0.02 c.c.
Pallidum (two strains) ..	0.375 c.c.	0.5 c.c.	0.02 c.c.
Microdentium	0.2 c.c.	0.3 c.c.	0.02 c.c.

It will be seen from the charts that the resistance of different species of Treponema and also of different strains of the same species (*T. pallidum*) seems to increase gradually until at the end of ten weeks (namely, five transfers) they were still able to grow very well in a medium which contained several (2–3.5) times the quantity of the arsenic compounds originally sufficient to restrain their growth completely. In case of bichloride of mercury the increased rate of tolerance was still more striking within a certain limit of concentration, but there was no further increase in resistance when the medium contained more than 0.5 c.c. of the 1:1000 dilution of this salt. The tissues which usually remain fleshy pink in color for several days became quickly discolorized into a dirty greyish black when mixed with the above concentration of $HgCl_2$.

The question of the duration of the acquired resistance to the drugs has not yet been studied a sufficiently long time to draw any conclusions, but the resistance has remained unmodified for at least three generations. It may be mentioned that *Spironema recurrentis* was carried through two generations in mice without undergoing any change in its acquired drug fastness.

Transmission of Spironema and Treponema to Man and Animals.—Under natural conditions the transmission of a blood-inhabiting Spironema to man or animals is effected through the bite of an infected blood-sucking insect. The transmitter in each instance is highly, if not strictly, specific, although other blood-sucking insects may also be infected by sucking the blood of an animal which is suffering from an infection with any of the pathogenic blood spironemata. These unnaturally infected ticks, bedbugs, fleas or lice are not good transmitting agents as compared with the natural carrier of the infection. That the spironema

in such non-specific insects can survive for some time can be shown when the disease is produced in a susceptible animal by inoculating it with the crushed material of the infected insects. It is possible, therefore, that an infection can be occasioned by smearing the excreta or crushed body contents of the infected insect over any defect of the epidermic layer of a susceptible subject. For example, in the case of *Spironema recurrentis,* both body-lice and bedbugs may be infected by sucking the blood of a patient suffering from the European relapsing fever, but the lice alone can transmit the disease to the next person they bite. Bedbugs are never known to spread the infection by their bites, although by crushing the infected bugs directly over a minute skin trauma (scratch, etc.), a person may become infected. A brief summary is given below of the natural intermediary hosts of different bearing spironemata, as well as certain experimental data bearing on the rôle of other blood-sucking insects and on the susceptibility of various animals to each Spironema.

Spironema recurrentis, the causative agent of the European relapsing fever, is naturally transmitted by *Pediculus corporis. Pediculus caputi* was found by Gonder to be incapable of transmitting the disease, although its body may contain the organism. The common bedbug (*Acanthia lectuaria*) may likewise harbor the Spironema for as long as 50 days,[130,131,132] but, according to the experiments of various investigators, does not spread the infection. The rat-louse (*Hematopinus spirulosus*) can carry the infection from rat to rat, while the monkey-louse does the same among monkeys. Breinl and Kinghorn,[133] as well as Neumann,[134] Manteufel,[135] and Sergent and Foley,[136] succeeded in transmitting the infection to rats with ticks (*Ornithodorus moubata*). Schuberg and Kuhn report a successful transmission by *Stomaxys,* the blood-sucking flies.

The infection can be transmitted subcutaneously as well as per os in experimental animals.

Infected organs fed to rats produce the infection in these animals, as shown by Uhlenhuth and Haendel [137] and others.[91] Manteufel considers the uninjured skin permeable to *S. recurrentis,* and Nattan-Larrier produced the infection per vaginam,

per penis, etc., in rats, while Gozony successfully transmitted the disease also by means of subcutaneous, conjunctival, and intestinal application of the Spironema. Schellack,[138] who obtained a positive result in one out of 28 experiments on rats, was able to demonstrate a microscopical defect of the skin at the point of entrance. The organism is experimentally transmissible to monkeys and from monkeys to rats, mice, guinea-pigs, and sometimes rabbits.

S. duttoni, the causative agent of the African tick fever, is normally carried by *Ornithodorus moubata*, as was recognized by Dutton and Todd,[139] and Koch.[139a] The last-named investigator discovered the spironema in the ovaries four to five days after the tick had sucked the infected blood. Carter[140] confirmed this finding, while Neumann[134] found the organisms in freshly laid eggs. Hereditary infection for one or more generations was shown to occur by the study of Dutton and Todd, Wolbach,[141] and others. The tick is infectious an hour after sucking and remains so as long as 90 days (Wittrock[142]). This author always found the Spironema in the infective ticks. *Ornithodorus savignyi* has been suspected of carrying the infection,[143] and Brumpt once succeeded in infecting a monkey by this tick. Robledo holds *Argas americanus* responsible for the spreading of *S. novyi* (the American type of relapsing fever) in Colombia, but this theory calls for further investigation. According to Breinl and Kinghorn,[144] the rat's fœtus may be infected through the placenta when a mother rat is inoculated with *S. duttoni*. The organism is experimentally transmissible to rats, mice, monkeys, guinea-pigs, and rarely to rabbits.

As has been briefly mentioned elsewhere, Leishman, Fantham, Hindle, and others assume a granular or coccoid phase in the life history of this and allied species and maintain that the spironema gradually undergoes granulation when it reaches the tick's body and multiplies in the Malpighian tubules and ovaries. The tick becomes infective after an incubation of 1–2 days at 37° C. Hindle[70] demonstrated the infectivity of the coxal fluid, in which he found numerous granules and some spironemata. This investigator thinks that the Spironema or infective granules in

the coxal fluid enter the body of persons through the wound produced by the bite of the tick. The infected eggs become infective after being incubated, as was demonstrated by Hindle by injecting the crushed material into the susceptible animals; Leishman [145, 146] found numerous spiral rods in the infected tick eggs when the latter were incubated for a few days at 35° C. Schubert and Manteufel showed the infectivity of the ticks to be lost when they are kept at a temperature below 22° C., but Gonder failed to find any such difference. Marchoux and Convy, Gleitmann, Wolbach, Wittrock, Kleine and Eckard, and others, believe that wherever infectivity is present, there are always to be found some typical spironemata, either in the tick or in its eggs.

Spironema berbera (the North African type) is carried by *Pediculus corporis* but not by *Argas,* the flea or bedbug [147, 148] (Sergent, Gillot and Foley). *S. carteri* is also transmitted by body-lice. In this case Mackie [149] found the organisms more numerous in the female lice than in the male; they are distributed in the mouth, stomach, and digestive tract. Mackie believes, however, that *Acanthia lectuaria* sometimes carries the infection.

Among the ticks which transmit spironema in cattle and sheep may be mentioned *Boophilus decolaratus* and *Rhipicephalus evertsi.* The virus is carried by heredity. The causative agent of chicken fever, *S. gallinarum,* is carried by *Argas persicus* under natural conditions, while other species (*Argas reflexus* and *Argas miniatus*) can transmit the disease experimentally (Schellack).[138] *Ornithodorus moubata* is doubtful, as Schellack [138] failed to produce the infection while Fülleborn and Mayer [150] claim a success with this organism. Schellack was able to produce the infection in 3 out of the 15 experiments performed by him on chickens by the percutaneous application of the infected blood. Feeding fowls with infected ticks may cause the infection. The organism is experimentally transmissible to ducks, geese, sparrows, canaries, and sometimes rabbits.

S. icterohæmorrhagiæ, the causative organism of Weil's disease, has been shown by Inada and his associates to be but rarely conveyed by direct contact, but no natural intermediary hosts have been discovered. The organisms are abundantly pres-

ent in the urine during the convalescent stage and they are fully virulent for guinea-pigs. According to the experiments of Inada and his associates, the Spironema as contained in the liver emulsion is capable of penetrating an apparently uninjured skin of the guinea-pig a short time after contact (5 minutes is sufficient to cause infection).* Therefore it is altogether possible to infect a person through direct contact with some of the excreta of a patient. The infection can be induced in guinea-pigs by introducing the infected material into the stomach after it has been previously neutralized with bicarbonate of soda. In regard to the Spironema found by Futaki and others at the site of or in glands adjacent to the rat-bite infection, we must assume that this represents the occurrence in rats of pathogenic Spironema which produces fever and other symptoms when transmitted to human subjects. Further investigation in this direction is most desirable. The organism is most easily experimentally transmissible to guinea-pigs. Monkeys, rats, and mice are less susceptible.

Prior to Futaki's work there appeared a report by Kitagawa and Mukoyama, who also found a spironema in the inflamed tissue of the bitten finger of a woman. By transmitting the tissue into guinea-pigs and white rats, these authors claim to have reproduced symptoms resembling the so-called rat-bite fever. In the smears of the kidney and liver taken from the dead animals, they found two types of spiral organisms, namely, in the guinea-pig tissues the refringens type and in the white rat the minute and short type. In examining the preparations kindly sent to me by the authors, I found their findings to be entirely correct, but the refringens type is more like *T. macrodentium,* and a large number of bacteria, such as fusiform bacilli and big rods, etc., were also present in the same preparations. As to the short type one can only say that its morphology is almost indistinguishable from that of *S. muris* or *S. microgyratum.* These organisms do not agree with the illustrations and description of the spironema reported some time later by Futaki and others. In the case of Kitagawa and

* Of eight guinea-pigs experimented upon only one escaped the infection which developed in from ten to twelve days with typical symptoms.

Mukoyama the local and general symptoms may have been due to a mixed infection by the oral flora of a rat.

Relapses of Infection.—The Spironema of relapsing and tick fevers also causes in man a well characterized type of fever accompanied by two attacks interrupted by a period of apyrexia lasting several days. During the apyrexia the blood is free of the parasites. But it is not at all rare for the recidive to be repeated more than once. While at the highest point of the fever the organisms are most abundant in the blood, they are also present in different organs. Dutton and Todd, Breinl, Leishman and Fantham believe that the spironemata are taken up by phagocytes within which they undergo transformation into the granular phase which in turn gives rise to the new generation of spironema. Balfour considered the intraglobular forms of *Spironema granulosa penetrans* (similar, probably identical, with *S. gallinarum*) as an asexual and the extracellular forms as a sexual phase. Fantham observed some extracellular granules which may start the relapse. Darling would hold the phagocytized spironemata within the endothelial cells of the liver responsible for the source of the recidive, as he found the organism to remain intact for some time during reconvalescence. Gabritschewsky sees in the surviving resistant specimens, which had been shielded from destruction in various organs, the progeny of the organisms producing the second attack. In the case of *S. carteri*, Mackie assumed the possible existence of an ultramicroscopic phase, as the serum taken from a patient at the apyrexic period is said to be infective in spite of the absence of any spiral forms. It may be remarked that to detect a sparse number of any Spironema under the microscope is one of the most difficult tasks, and one is very liable to overlook the organism.

T. pallidum and *T. pertenue* are the only pathogenic varieties among the group. In the case of syphilis our knowledge is quite complete, so far as the mode of transmission is concerned. On the other hand, much still remains to be learned regarding the manner in which yaws is communicated from person to person. Probably, like syphilis, its infection is spread by direct contact with a patient or any object which, after having been in contact

with a patient, harbors the live organisms; although the possibility of transmission through flies, mosquitoes and ticks is not excluded. Castellani and Chalmers [151] quote an instance in which a fly which sucked on a yaws papule infected a monkey whose eyebrow was scarified. Modder [152] assumes transmission of yaws by ticks (Argas and Ixodes) in Ceylon. It is said that vaccination and wet nursing spread the infection. In order to facilitate their entrance into the human body both organisms need only a microscopical defect of the epidermis.

After penetrating the skin or mucous membrane, *T. pallidum* elicits a local reaction characterized by the circumscribed round cell infiltration known as chancre (primary lesion), then several weeks later, at about the time when the chancre recedes, it proceeds to enter the adjacent lymph-glands and general cutaneous and mucous membrane tissue, producing roseola, papules and flat condyloma. At this period the organisms invade almost every tissue, producing the so-called secondary symptoms. Periostitis, meningitis, iritis, laryngitis are very frequently observed. One of the most constant symptoms is the Wassermann reaction in the blood serum.

After a period of several months longer, during which the secondary manifestations abate, another period known as the tertiary stage may supervene accompanied by still deeper tissue destruction caused in the organism than at any previous stages. It affects skin, bones, visceral organs, cardiovascular system and central nervous system. The disease may be progressive or marked with alternate activity and latency. Yet in the latent period repeated abortions may occur. From the time of infection until the central nervous system is affected (general paralysis, tabes), the average period of latency of the disease is from eight to twelve years. During the tertiary stage the lesions are often gummatous and affect the connective tissue, muscles and blood-vessels, while in cases of general paralysis and tabes the parasites diffusely pervade the parenchyma.[153, 154, 155, 156] This form is a syphilitic parenchymatous encephalomyelitis. In acquired syphilis, *T. pallidum* has been demonstrated in every syphilitic condition. It was first demonstrated in the primary and secondary

lesions by its discoverers, Schaudinn and Hoffmann; in liver gumma by Schaudinn; in aortitis by Wright and Richardson,[157] Schmorl,[158] and Reuter;[159] in arteriitis cerebralis by Bender;[160] in heart muscles and pancreatitis by Warthin;[161] in adrenal glands by Hoffman,[162] Jacquet and Sezary;[163] in nephritis by Hoffmann;[166] in the cerebrospinal fluid by Hoffmann,[165] Nichols and Hough,[166] and Suzary and Paillard;[167] in the blood during the secondary stage by Uhlenhuth and Mulzer;[168] in paretics by Graves,[169] in interstitial keratitis by Igelsheim;[170] in cerebral gumma by Dunlap;[171] in the paretic brains by Noguchi and Moore,[172] Marinesco, and Miner,[173] Levaditi, Marie and Bankowsky,[174] Mott, Rosanoff,[175] Tomasczewski and Forster,[176] Wile,[177] and others; in spinal cord by Noguchi,[178] Versé,[179] etc.

It should be mentioned that the first demonstration of *T. pallidum* in sections of tissue from acquired syphilis was accomplished by Bertarelli and Volpino [180] by means of their silver impregnation; a method which has since been superseded by a similar procedure amended by Levaditi.

In congenital syphilis the number of organisms present in the different organs and in different fœtuses varies greatly. In some it may be extremely tedious to demonstrate the organisms, in others the whole fœtus may be thickly interwoven with the intertwining nets of treponemata. The favorite site of invasion is the liver and skin, although stomach, intestines, adrenals, kidney, spleen, heart muscles, pancreas, bone-marrow, lymph-glands, thymus, testes, ovaries, and brain have been shown to contain the parasites, even in large numbers in certain instances.[181] The placenta and navel cord are also affected. For the first demonstration of the organisms in congenital lues, we are indebted to Levaditi,[182] who introduced his well-known silver impregnation method for this study. According to personal experiences in connection with syphilitic infants who lived several days after birth, the number of pallida present was always very small and it sometimes required many hours' search to find a single specimen. A striking difference between syphilis and yaws is the absence in yaws of visceral affections and of the nervous involvement. Much yet remains to be investigated with regard

to the relationship between syphilis and yaws, the causative agents of which bear so great a morphological, and to a certain extent, a biological resemblance toward each other.

The transmissibility of syphilis to animals was long the subject of study by earlier investigators, but the first conclusive experiments in this connection were furnished by Metschnikoff and Roux,[183] who succeeded before the discovery of *T. pallidum* by Schaudinn in producing the primary and secondary lesions in chimpanzees. It was also shown that these lesions were transferable to further series of animals. Immediately after the discovery of *T. pallidum* in human syphilitic tissues, Metschnikoff and Roux [183] found the same organism in experimental syphilis, thus closing up the first link of the chain of evidence which was to prove the specificity of the organism for syphilis. They also infected macacus monkeys with the virus derived from chimpanzees. Soon afterward Schultze [184] and Bertarelli [185] produced syphilitic keratitis in rabbits, while Parodi [186] selected the testes (intratesticular) to transmit the human strain to the rabbit. This work has been extended and elaborated by later investigators, particularly by Neisser,[187] Hoffmann, Locke and Mulzer,[188] Uhlenhuth and Mulzer,[189] Grouven,[190, 191, 192] Nichols,[193] Tomasczewski,[194, 195, 196, 197] and others. In monkeys the best site for inoculation is the eye-brow, while in rabbits intratesticular, scrotal, intraocular and intracardial inoculations were recommended. For the purpose of keeping up the pallidum strain the intratesticular mode is preferable, especially when it is desired to obtain a pure material for cultivation (Uhlenhuth, Noguchi); but in case of utilizing the lesions in order to determine the effect of a therapeutic agent, Hata [128] recommends the scrotal chancre method (introduced by Tomasczewski) wherein he is supported by the experience of Brown and Pearce.[198] With the purpose of causing a generalized syphilis in the rabbit—which animal is usually refractory to the systemic pallidum infection—Grouven reports the intracardial introduction of a large quantity of the pallidum in half-grown rabbits. My own numerous attempts to produce generalized syphilis by this method completely failed, probably owing to the difference in the strains employed. It may be mentioned, how-

ever, that with certain pallidum strains symptoms similar to human secondaries or tertiaries could be produced by means of intravenous or intratesticular inoculation. I have a few times observed iritis, keratitis, and squamatous or ulcerative skin lesions, in the last of which the pallidum could be demonstrated. Nichols and Hough [166] were the first, however, to isolate a strain from a case of nervous recidive which constantly invaded the cornea, even before the local symptoms (testis) commenced to appear. This strain has most persistently caused keratitis and choroido-retinitis in rabbits. This phenomenon led Nichols to assume that the strain possessed a highly invasive character.[199] The patient from whom this strain was obtained died several months later of a rapidly progressive form of meningo-encephalitis, and the duration of the infection (from the time of the chancre to death) was very short. Nichols, therefore, considered that this case was explained by the character of the strain. Reasoner [200] also obtained a strain from a rapidly fatal case which was characterized by the early production of choroiditis in rabbits. In some rabbits the choroiditis was the only symptom in spite of its being introduced into the testis or vein. While studying ten different strains of Treponema pallidum I was once struck with the constancy with which the various types were associated with certain distinct characters of the lesions produced in rabbits. For example, I could discern the differences among different strains in the width, length, and number of curves to a given space, etc. I divided these strains into a thick, a thin, and a medium type. The differences were great enough to enable me to identify different strains as belonging to any one of the three types.[201] In a series of passages covering a period of about one year and a half, it was found that the thin type produced a soft, diffusely swelling orchitis within 10 to 14 days and did not form any definite nodules even after six weeks. On the other hand, the thick type produced a hard, circumscribed nodule of varying size within about six weeks. Its development was unusually slow and the nodule remained for several more weeks. The character of the syphiloma produced by the medium type was a large, moderately firm orchitis, which started to be palpable at the end

of about four weeks. As will be mentioned later, these three type strains were cultivated in an artificial medium and were found to retain their morphological characteristics unchanged. Again, my experience with the two paretic strains of Treponema pallidum transmitted from human brains to rabbits' testicles (using 36 rabbits for six specimens of brains) showed me that they were of lower virulence than the ordinary chancre strains in my possession, as they required 97 and 102 days, respectively, before the lesions could be definitely demonstrated.[119] With the usual skin strains four weeks' incubation is the average. Wile [177] recently reported a successful transmission of the pallidum from the living paretics to rabbits' testicles (using one rabbit for six specimens of brains) in which the lesions appeared within 14 days, and he concluded that the paretic strains were more virulent than the ordinary strains. It may be recalled that the persistent endeavors to produce syphilitic orchitis in rabbits by means of the paretic brains was not limited to a few investigators. Tomasczewski and Forster,[202] who in 1913 performed the Neisser-Pollack puncture on 62 cases at the University Institute in Berlin and found numerous examples of the motile pallida in 29 cases of the removed material, inoculated a large number of rabbits. Their results were uniformly negative. Marie, Levaditi and Bankowsky, Marinesco, Mott, and others also failed to obtain a single positive result. Another interesting feature characteristic of the strain obtained by Wile [203] is the readiness with which it at once adapted itself to an artificial culture medium generally known to be unsuitable for the purpose of obtaining an initial growth with any strain which is transmitted to the rabbits' testicle. As I have pointed out on several previous occasions, a solid medium consisting of fresh tissue, ascitic fluid and agar is not suitable for such a purpose, and this fact has been confirmed by numerous investigators (Zinsser and Hopkins, Uhlenhuth, etc.).

It will be incomplete if we pass on without reviewing the interesting observations of Graves,[169] who succeeded in infecting a certain number of rabbits by injecting the blood of paretic patients. Graves obtained the blood in small glass ampules (sterile) which were immediately sealed. The different speci-

mens were put in an incubator at about 37° C., and after a number of days the contents of these ampules were inoculated into the testicles of rabbits. Although the majority of the inoculations were negative he found a strain developing in one of the animals. Morphologically, the organisms were the typical pallidum and produced local as well as generalized reactions (ulcerative lesions near the nostrils, anus, prepuce, vagina, etc.) wherein the organisms were demonstrated. The incubation period of average duration is about 3–4 weeks. This strain was characterized by the early appearance of keratitis in rabbits. The observation of Graves furnishes us with a problem, *viz.*, the fact that the sample of paretic blood sealed in a tube and left many weeks and months at an indifferent temperature was still capable of infecting a rabbit with such extreme severity. Yet Graves never succeeded in cultivating any strain of such examples; neither could he demonstrate the presence of any definite pallidum. Therefore, as Graves seems to think, Treponema pallidum must possess a stage of its life-cycle which is still little understood by us. Can there be a resistant form which remains dormant for years until favorable conditions are secured? Clinical evidence has suggested this idea to certain syphilologists (Pollitzer). Personal experiences with cultivated strains of Treponema pallidum do not justify my assuming the existence of a resistant form, except for the fact that the pallidum under cultural conditions is one of the most viable organisms. In suitable media it survives over one year when kept at 37° C., and it is not impossible that under naturally favorable conditions it may remain dormant for many years.

Several other animals besides monkeys and rabbits are susceptible to the disease. In dogs and sheep (Bertarelli, Hoffmann, Brüning), in guinea-pigs and goats (Bertarelli), and in cats (Levaditi and Yamanouchi) specific keratitis has been produced. Testicles of guinea-pigs (Truffi, Tomasczewski, W. H. Hoffmann, Uhlenhuth and Mulzer) and goats (Uhlenhuth and Mulzer) are also susceptible to infection by Treponema pallidum. Schereschewsky reported a scrotal chancre experimentally produced in a pig.

Treponema pertenue has been successfully transmitted into

monkeys by Neisser, Baermann and Halberstädter [204] with skin papules, and by Castellani [205] with a punctate of the spleen of a patient. Nichols [206] transmitted it from man to Macacus rhesus and then from the latter to the rabbit. In the rabbit's testicle it produces a hard induration much like a syphilitic chancre. The parenchymatous orchitis finally extends over to the tunica and scrotum, in which an extensive ulcerative indurated lesion results. When inoculated to the eyebrows of Macacus rhesus the yaws organism produces highly destructive ulcerative papules which may remain unhealed for many months. In these lesions Treponema pertenue can easily be demonstrated. Halberstädter [207] observed a generalized eruption in an orang-utan four months after inoculation. In lower monkeys the lesion remains localized and heals in from three to thirteen weeks; sometimes it may result in a serpiginous recidive which tends to become diffuse.

Filterability of Spironema and Treponema.—According to the experiments of Novy and Knapp,[18] Spironema recurrentis and S. duttoni pass in one form or another through the pores of Berkefeld filters, the walls of which were either previously shaved off to a thickness of 1.4 to 2.5 mm. or left intact (4.2 mm.) as the filtrates obtained by this procedure were able to produce in susceptible animals a slight infection accompanied by sparse spironemata appearing in the blood. The scarcity of the organism is ascribed to the presence of immune substances in the filtrate which was simultaneously introduced. Breinl and Kinghorn [132] obtained similar results with unmodified filters. In neither instance did the infective filtrates contain the Spironema in its spiral form. Their experiments tended to suggest a filterable phase in the life cycle of this organism. Todd and Wolbach [208] report the successful filtration of the organism through the Berkefeld filters N and V by pressures of fifty to ninety pounds to the square inch. Under these conditions the organism traversed the tortuous pores of the filters and was seen to have retained its usual spiral form when it appeared in the filtrate. Todd succeeded in finding the organisms in the filtrates of one experiment into which the control bacteria did not pass. Wolbach found the Spironema in the act of passing through the pores,

by preparing a thin section of the filter which had been employed for the filtration. He is of the opinion that the infectivity of a filtrate is due to the presence of the regular organism and not to that of filterable granules, as is assumed by others. C. Fraenkel failed to obtain an infective filtrate with any filters whatever.

Spironema icterohæmorrhagiæ was found by Inada and his associates [79] to pass through the Berkefeld filters, grades V and N. Out of 28 experiments the filtrates were found to be infective for guinea-pigs 15 times. It is not stated whether the filtrate contained the spironema in a regular form. Huebener and Reiter [209] also report the filterability of the virus of Weil's disease prevalent in Germany. Since they claim Spirochæta nodosa, found by them, to be the etiological agent, the same organism must be considered filterable. It passes through the Berkefeld filters V and N. As mentioned elsewhere, Spironema nodosum (Spirochæta nodosa) is probably the same organism as Spironema icterohæmorrhagiæ (Spirochæta icterohæmorrhagiæ) discovered a year earlier by Inada.

Treponema pallidum and Treponema pertenue are unable to pass through any bacteria-proof filters when filtered by the usual processes (application of a vacuum or a positive [compressed air] pressure). Metschnikoff, Klingmüller and Baermann,[210] Casagrandi and de Luca,[211] and many others established this fact in the case of syphilis, and Castellani in the case of yaws. On the other hand, the pallidum can grow through the pores of the Berkefeld filters,[57] grades V and N, and appear in the filtrate when provided with favorable cultural conditions for several days. On the fourth day the young forms commence to appear in the fluid which collects in the empty tube which is fitted up to receive the drops that fall by spontaneous diffusion without suction or pressure. This phenomenon, which was first noticed and utilized by myself when obtaining a pure culture from mixed cultures, has since been confirmed by Nakano [212] and others.

There are yet other spiral organisms which are of great interest from the standpoint of filterability. Thus, Wolbach and Binger described Spirochæta elusa [213] and Spirochæta biflexa,[214] which they obtained in a filtrate of stagnant water taken from

the shores of a fresh water pond in the vicinity of Boston. The first was cultivated but the second was not. With the culture of S. elusa, which measures about 0.5μ wide and 20μ long with an average of six to eight curves, they were able to demonstrate the organism in the filtrate within about fifteen minutes. The filtration was made by suction with the Berkefeld filters, V and N. The organism is provided with one terminal flagellum at each end and is extremely motile. Spirochæta biflexa was a much more delicate organism. Another filterable organism, morphologically considered a "spirochæta" in the loose sense of the term, was obtained by Wolbach and his associates from human fæces. In explaining the filterability of these rather coarse spiral organisms, which are larger than many bacteria, Wolbach considers their plasticity to be one of the important factors.

Cultivation.—Only a comparatively limited number of "spirochætes" have been cultivated on artificial media. Of the free-living varieties Spirochæta plicatilis was cultivated by Zuelzer [22] in a flask containing ¾ liter of stagnant lake water and ¼ liter of water to which a certain amount of hydrogen sulphide had been added. According to this procedure the flask is hermetically (anærobic) sealed after the inoculation and hydrogen sulphide occasionally introduced. The rôle of H_2S is to produce sulphur by oxidation ($H_2S + O = H_2O + S$). By this means the organism can be kept in culture for an indefinite period. Wolbach's Spirochæta elusa was cultivated on a hay infusion (ærobically) where it propagates indefinitely. This organism is not allied to Ehrenberg's organism, but appears more like a Spirillum. No culture has been obtained of the molluscan cristispira. Of the Spironema group several varieties have been cultivated. Attempts at the cultivation of Spironema novyi by Norris, Pappenheimer and Flournoy [215] were partially successful in that these investigators were able to notice a definite multiplication of the organism in human or rat citrate blood at room temperature within 24 hours, a second generation cultivated in a similar medium bringing about the same increase the next day. A third generation was not obtained. Occasional multiplication of the Spironema in a defibrinated blood had previously been shown by

earlier investigators (Lachmann, Albrecht, Gerhardt). Cultivation of the blood Spironema in the strict sense of the term was achieved by the writer [216, 217] for the first time by employing a culture medium containing a piece of fresh tissue (rabbit's kidney, etc.) and ascitic fluid (10 to 12 cm. deep). This medium provides a condition that I proposed to designate as an *aërotropic anaërobiosis;* that is, a strictly anaërobic state is produced around the base of the fresh tissue, while the top of the ascitic fluid column has access to a certain quantity of oxygen. The whole medium may be covered with a layer of sterile paraffin oil in order to prevent evaporation of the fluid, and this regulates at the same time the amount of oxygen admitted. When the medium is inoculated with a minute quantity of the Spironema-containing blood and then incubated at 37° C., the organism multiplies steadily until every field will show numerous motile specimens which may occur singly, in pairs, or in chains of three or more individuals. The height of multiplication is reached within four or six days, and a sudden degeneration of the organisms sets in on the seventh to the ninth day. By making subcultures on the fourth or fifth day the culture can be carried on indefinitely. It was found that the success or failure greatly depends upon the suitability of the ascitic fluid samples. A sample which forms a loose fibrin web with the fresh tissue (rabbit's kidney) within 24 hours at 37° C. gives the best empirical results. The addition of glucose or pepton seems to hinder the growth of the Spironema, and sterilization by filtration or fractional heating also impairs the nutrient value of the medium. Invasion of the culture by any other bacteria quickly destroys the culture; in other words, no mixed culture has been obtained. So far I have been able to cultivate S. recurrentis, S. novyi, S. duttoni, S. kochi and S. gallinarum by the same method. Plotz [218] successfully applied this method in order to obtain a culture directly from patients in Bulgaria suffering from the European relapsing fever.* According to Hata,[219] instead of the fresh tissue and ascitic fluid, a medium consisting of a piece of blood coagulum and of the serum of the horse may be satisfac-

* Personal communication.

torily used for cultivating S. recurrentis. At the temperature of 26° C. the culture in this medium remains actively motile for a month (Hata). None of the spironemata causing septicæmia in man or birds have been cultivated on a solid medium, and nothing is known about such colonies. In a fluid medium they produce a diffuse opalescence but no definite change of the medium is noticed. No odor or gas or change in the reaction has been detected in the culture. Their virulence remains unattenuated for many generations of artificial cultivation.

From the oral cavity S. vincenti and several spiral organisms have been obtained in pure cultures by various investigators. Tunnicliff [220] considers S. vincenti and Bacillus fusiformis to be identical, but in different phases of development and under varying conditions. All these organisms are strictly anaërobic and can be cultivated by the usual anaërobic methods on solid media (glucose agar with or without animal proteids). They form definite colonies comparable to any other bacteria, and some are putrefactive or acid-producing organisms. I am inclined to regard them as allied to Spirillum rather than to Spironema.

Spironema icterohæmorrhagiæ has been cultivated by Inada [79] and Ito in the same medium as was originally employed by the writer for the cultivation of S. recurrentis, S. duttoni, S. gallinarum, etc. Ito [221] later succeeded in cultivating the organism on agar or gelatine containing human or guinea-pig defibrinated blood in the ratio of equal parts, or one to two, of blood and agar or gelatine. The organism is said to grow readily on these media at a temperature of 26°–37° C. A good growth takes place even at room temperature. Characteristic cultural features, such as gas or odor production, colonies, turbidity, etc., have not been recorded. In the fluid as well as in the solid media no visible growth was obtained. The culture is said to remain virulent for many generations and lives over one month in a solid medium when kept at about 15°–26° C.

In proportion to its clinical importance Treponema pallidum has ever since its discovery in 1905 been the subject of correspondingly more numerous investigations. Volpino and Fontana [222] (1906) observed a temporary increase of the organisms

after a piece of syphilitic tissue had been put in human serum or defibrinated blood and then incubated at 37° C., but no culture was obtained. Lebailly [223] claims to have kept alive the pallida in the syphilitic fetal tissue for 15 days when put in human serum at 37° C. Levaditi and McIntosh [224] inoculated the inactivated human serum with the expressed serum of a syphilitic lesion of a monkey containing a few pallida and, after sealing it in a collodion sac, introduced it into the peritoneal cavity of a monkey. It was taken out after a month and was found to contain numerous motile pallida along with certain contaminating bacteria. The contents of the sac cultivated *in vivo* could be successfully transferred from one sac to another for many passages with the same result. The impure pallida cultivated by this method were avirulent for monkeys. Mühlens and Loehe [225] failed to confirm the above findings. In 1909 Shereschewsky [226, 227] claimed to have succeeded in starting an impure culture of Treponema pallidum by implanting a semi-solidified, clear horse serum with a piece of chancre or condyloma inserted several inches below the surface. The serum commenced to liquefy around the tissue and within several days more liquefaction took place. On examining the fluid or solid medium about the tissue, he found very numerous actively motile spiral organisms resembling the pallidum. Some of them were coarser and less regularly curved and looked like S. refringens. An enormous number of cocci or bacilli were also present. The culture gave off an intensely offensive odor. Subcultures were carried on indefinitely. The impure culture was avirulent for experimental animals. About the same time Mühlens [228] obtained a pure culture of an organism from a syphilitic lymphadenitis of man by first using Schereschewsky's medium and then transferring the culture to another kind of solid medium consisting of horse serum and agar. In the latter he succeeded in purifying the treponema from the contaminating bacteria. Notwithstanding the fact that the organism was derived from a material in which the pallidum would be the only small treponema, and in spite of its great resemblance to the pallidum, Mühlens's culture has certain characteristics which, as will be seen later, render the organism distinguishable from

the pallidum cultures which were obtained by others (Noguchi,[229, 230] Sowade,[231] Tomasczewski,[232] Baeslack,[233] Zinsser, Hopkins and Gilbero [234]). Thus the organism isolated by Mühlens was avirulent, produced a strong odor and could grow from the beginning in a horse serum agar without the addition of any fresh tissue. W. H. Hoffmann [235] (1910–1911), a co-worker of Mühlens, obtained several strains which were identical with that of Mühlens, except for the fact that he was able to produce in the rabbit's testicle a somewhat acute or subacute inflammation by injecting a large quantity of solid culture.[236] His description of the experiments leaves the syphilitic nature of the lesion indefinitely established. The extract of the organism acted as an antigen in the Wassermann reaction, as was also shown by Schereschewsky [237] in the case of his impure culture; but Mühlens as well as Schereschewsky obtained similar results when extracts of other bacteria were used. Recently Zinsser and Hopkins have confirmed the non-specific nature of so-called antigens in this type of complement fixation. Bruckner and Galasvesco [238] (1910) and Sowade [231] (1911) reported the successful inoculation of rabbits by means of their impure cultures (Schereschewsky medium) given intratesticularly and intracardially. Sowade claims to have produced generalized syphilis by the intracardial injection, into half-grown rabbits.

During 1910–1911 I was engaged in cultivating T. pallidum.[57, 120] Unlike the previous investigators, I had chosen the testicular syphiloma of rabbits as the material for cultivation, for the reason that in this material we have a constant and unlimited supply of a practically pure pallidum and as many strains simultaneously as one desires to try. Besides, the rabbit strains being already acclimatized to the animal, this would more readily take on when a culture derived from this source is to be tested for its virulence. After unsuccessful attempts to cultivate the pallidum in all the various media previously reported suitable for cultivation of the pallidum, and a large number of culture media, and conditions having also failed, the following two methods were found to yield a positive growth of the organism on an artificial medium. As has been mentioned elsewhere, neither method is a

perfect one, and only a limited percentage of attempts is ever successful. The inconstant results are due partly to the different resistance offered by various strains to the artificial cultivation, and partly to certain still unknown factors which enter into the composition of the media. At all events, the greatest difficulty in cultivating the pallidum is to obtain the first growth. As the number of generations increases, the organism acquires an easier growth, and after a period of years of life in the culture the organism becomes quite saprophytic and may grow even without the addition of fresh tissues. The strict requirements demanded by anaërobiosis and by the reactions and compositions of the media become more and more lax until the culture may adapt itself to a great many cultural conditions. The two methods above mentioned are (1) a fluid medium consisting of a suitable sample of ascitic fluid or sheep serum water (His) with the addition of a piece of freshly removed kidney or testicle from a normal rabbit; and (2) a solid medium consisting of a mixture of ascitic fluid and agar with the addition of a piece of fresh tissue as above described. The use of the fresh tissue seems to offer twofold advantages. First, as an oxygen absorbent as originally recommended by Th. Smith,[239] and secondly, as a source of nutrient substances needed for the pallidum. The first method (fluid medium) is applied exclusively for the cultivation of the testicular pallidum from rabbits, and the second (solid medium) is only used to cultivate the impure material derived directly from human syphilitic tissues. The first method requires an anaërobic apparatus, as a complete removal of oxygen from the atmosphere in which the cultivation is to be carried out is essential, while for the solid medium a layer of sterile liquid paraffin poured on the top of the culture medium suffices to prevent evaporation and possibly to minimize the diffusion of oxygen into the medium. The requisite anaërobiosis is produced by the fresh tissue which lies at the bottom of the tube. I shall not enter into any technical details, but suffice it to say that nearly a dozen strains were obtained within the last few years by the use of these two methods. The strains obtained by means of the fluid medium remained

for many generations unadaptable to the solid medium to which they finally grew. On the other hand, the strains grown on a solid medium could readily be made to grow when suitable conditions were provided.* Impure pallidum cultures in a fluid medium can be purified by allowing the pallidum to grow through the pores of a Berkefeld filter. Before the associating bacteria passes, the pallidum will appear in the filtrate (by gravitation), probably on the fourth or fifth day. Some of the strains of T. pallidum obtained by these methods were virulent to rabbits and monkeys when tested within a few months. The lesions produced were typical in every respect, although once the organism had entered the animal body it resisted recultivation just as much as before the first cultivation. In this respect they differ from the strains of W. H. Hoffmann,[236] who was able to cultivate the organisms back from the lesions into the horse serum agar without the addition of any tissue. His strains produced a strong offensive odor when recultivated. The strains cultivated in my laboratory did not, and still do not, give any offensive odor such as is described by Mühlens and W. H. Hoffmann. As has been stated, no growth could be obtained without the aid of fresh tissue during the first year after these strains were isolated. Nor could they be induced to grow on a plain horse serum agar of Mühlens or semi-coagulated horse serum of Schereschewsky. Since attention had been called to the differences which existed between the cultures of Mühlens and Hoffmann and my own cultures, later investigators gave special attention to detecting any possible production of a peculiarly offensive odor. Sowade, Tomasczewski, Baeslak, Nakano, Zinsser, Hopkins and Gilbert failed to find any such characteristic odor in their strains. Erich Hoffmann [240] considers that the cultures of Mühlens and W. H. Hoffmann either contained the pallidum and a second odor-producing organism or were not Treponema pallidum at all, since there exist certain pallidum-like, easily cultivated, saprophytic treponemata which in pure cultures produce a strongly offensive odor (T. microdentium, T. mucosum, *vide infra*).

That the cultivated strains of Treponema pallidum gradually become tolerant to various media and conditions had been strik-

* Fluid medium method.

ingly demonstrated by the recent investigations of Zinsser, Hopkins and Gilbert.[234] Thus the investigators found that a pallidum strain which they had isolated by the original fluid culture method, described by me, gave a good growth after the tenth generation in fluid media containing different kinds of autoclaved tissues of rabbits and a mixture of slightly acid meat infusion broth with heated sheep serum. This strain likewise grows well in symbiosis with staphylococci, streptococci, Micrococcus candicans and bacillus fæcalis alkaligenes added to the sheep serum agar mixture without tissue. Addition of dead staphylococci had the same effect as symbiosis. The gelatinized horse serum or sheep serum with or without the tissue proved to be a good medium for the growth of this strain. They have obtained the same results with two other strains which were isolated in my laboratory several years ago. The fact that during the first few years after isolation all my pallidum strains failed to grow on various media similar to those now successfully used by Zinsser, Hopkins and Gilbert seems to indicate that rigid parasitic properties of the organism have gradually deteriorated, due to the artificial cultural conditions, some undergoing the changes more abruptly than others. It is not at all improbable that in time the saprophytized strains of Treponema pallidum will adapt themselves to still simpler ordinary culture media. It is to be desired that efforts be directed toward improving the condition under which the organisms can be kept as nearly natural as those in living tissues, since the results obtained with completely denatured material might be different from those derived with the less modified material.

In summing up the pallidum cultivation one may say that the methods hitherto proposed are still imperfect and that much patience is still demanded in order to isolate a strain. Some strains remained persistently unamenable to cultivation in my hands. The strains isolated by Mühlens may have been Treponema pallidum; but there was no way of proving this, as his culture, which was avirulent, possessed certain properties inconsistent with those of the pallidum as subsequently defined by other investigators. The first instance, therefore, of a successful culti-

vation of Treponema pallidum was that which was carried out at the Rockefeller Institute in 1910–1911, in which were brought out not only the demonstration of the pathogenicity of the organism isolated but also the studies of other biological characteristics of the culture. Treponema pertenue [241] was also successfully cultivated in 1911 by the same method as that given for the cultivation of the pallidum. The material used for this work was in the form of a testicular lesion of experimental yaws in the rabbit and was supplied by Captain Nichols. The organism possessed the same cultural characteristics as the pallidum, but it was probably slightly thicker and less regularly curved. Preliminary attempts to produce lesions in rabbits ended negatively, and the strain was lost before further comparative studies could be undertaken.

Several spiral organisms were isolated from unclean lesions around the genital regions. Spironema refringens [242] and Treponema calligyrum [104] (from a condyloma) were cultivated by me in the pure state by methods similar to those used for the pallidum and pertenue. Levaditi and Stanesco [243] obtained impure cultures of S. gracilis and S. balanitidis by means of gelatinized horse serum. A spiral organism, *S. phagedenis,* was also obtained by me in a pure culture from a phagedenic ulcer on the genitalia of a woman, but its systematic affinity is quite uncertain.[95] T. calligyrum is slightly coarser than the pallidum but is apt to be mistaken for the latter in cultures. It grows easily in tissue-free media.

From dental deposits of normal oral cavity Treponema macrodentium and T. microdentium [102] were cultivated in the pure state by means of the same methods, and Treponema mucosum [103] from the scraping of the pyorrhœal gum. The microdentium and the mucosum appear very similar, but can be distinguished by the production of a thin, but tenacious, mucin in the culture of the latter. When the culture gets old, both of these give a strong, somewhat offensive odor. This faculty to decompose the proteids (thus causing a slight turbidity in the fluid media) makes this culture readily distinguishable from the pallidum cultures, because the latter do not produce such an odor. Morphologically they bear a great resemblance to the pallidum,

although their curves are set somewhat more closely than in the pallidum. The macrodentium is more difficult to cultivate and, according to my experience, requires fresh tissue in the culture media. Morphologically it is coarser than the pallidum and its serpentine movements and irregular, stretchable, and wider curves are characteristic enough to distinguish this species from other varieties. It does not produce an odor.

Mühlens and Hartmann [244] succeeded in 1906 in obtaining a pure culture of S. dentium in the horse serum agar medium of Mühlens. In their culture they recognized a minute form of Koch's S. dentium type and another which approached the dimension of Hoffmann-Prowazek's S. media type. They suggested the possibility of these representing different stages of development or even a sexual differentiation. It appears as though their so-called pure culture may have contained more than one species. No admixture of tiny and coarse forms has been observed in the culture of the macrodentium or microdentium. The dentium culture of Mühlens produced a strong offensive odor.

Immunity and Immunization.—The very name, relapsing fever, suggests the possibility of the development of some sort of protective power in the infected hosts against a third attack. In fact, the second attack is often milder than the first and a third relapse is rare. Persons who have had the fever are usually immune to subsequent infection for a period of several years. The same is true of the African tick fever, although repeated recurrences are more frequent in this instance. Susceptible animals such as monkeys, mice, and white rats, enjoy a period of immunity extending over about three months after recovering from the second attack. In rats no relapse has been observed. In the fowl and geese spironematosis similar immunity follows recovery. The studies of various investigators, especially Gabritschewsky, Pfeiffer, Novy and Knapp, Manteufel, Marchoux and Salimbeni, Levaditi and Manouélian, Prowazek, Neufeld and others, have contributed in explaining the mechanism upon which the immunity depends. Gabritschewsky [245] demonstrated the presence of a specific antibody against S. recurrentis in the blood of convalescent patients by mixing it with the spironema-contain-

ing blood *in vitro*. The destruction of the organism occurred within a short time when the mixture was kept at a temperature of 37° C. This author considered that the development of a germicidal substance in a patient's blood was the cause of the crisis and subsequent immunity. He also recognized the appearance of a similar specific immune substance in the geese recovering from the attack of S. anserina. The convalescent recurrentis blood had no effect upon the organisms of goose fever, and the anserina blood did not affect the organisms of the relapsing fever. The phenomena observed were agglutination, immobilization and dissolution of the organisms when mixed with their correspondingly specific bloods. Gabritschewsky produced an immune serum by injecting the horse with the spironema-containing blood. It was tested by Löwenthal in 83 cases and in 39 cases (47 per cent.) no relapses occurred, while in 140 untreated cases 65 had three attacks (46.5 per cent.). Novy and Knapp [18] confirmed and greatly extended the experimental parts of Gabritschewsky's work and pointed out that the protection afforded by active as well as passive immunity is not wholly dependent upon the germicidal property but also upon the immune bodies, since a comparatively weak germicidal blood may protect the animal against the infection in small quantities. Besides, Novy and Knapp hold the rôle of the phagocytes (mononuclear, but not polynuclear) to be very important, as they ingest the dead as well as the enfeebled spironemata under the influence of immune bodies. Levaditi and Manouélian [246] suggest the existence of an opsonin in this phenomenon. Manteufel [247] believes the lysis of the spironemata in the immune serum to be due to the co-operation of complement and a specific amboceptor. The rapidity and intensity with which the spironemata are destroyed within the peritoneal cavity of actively or passively immunized rats is variable. In the peritoneal cavity of a hyperimmunized animal, the organisms become granular in from 2 to 5 minutes, while in rats recently recovered from the attacks the organisms are ingested in 15 minutes. In passively immunized rats the spironemata are first agglomerated and temporarily immobilized, and this is followed by the appearance of some leucocytes on the scene; but the effects of the immune

substances gradually wear off within about an hour. The leucocytes disappear in 30 minutes (Novy and Knapp). The germicidal and bacteriolytic actions are parallel. The duration of passive immunity in rats is less than 40 days while that of the active immunity lasts nearly four months. Novy and Knapp succeeded in preparing in rats by means of hyperimmunization a powerful immune serum which contained in each cubic centimetre 500 immunity units; that is, 0.002 c.c. of the serum was able to protect the rat against 0.1 c.c. of the infective blood showing 10–50 spironemata per field (2 mm. objective). In ordinary recovered rats there were only about 2 immunity units per cubic centimetre. The use of the immune blood from a hyperimmunized rat prevented the infection in the rat and cured it on its onset, but a greater amount is found necessary in order to obtain similar results in monkeys and mice as these animals are subject to a relapse after the treatment. Novy and Knapp suggested the inoculation of the spironema during the apyretic period in order to increase the amount of immune principles in the victim's system and thereby ward off a relapse. They found an interesting phenomenon, *i.e.,* the injection of too much immune blood proved to be less effective than a moderate quantity. This was explained by assuming the production of a specific precipitin which acted as an anticomplement. In regard to the use of hyperimmunized blood serum in human relapsing fever, they calculated that about 375 c.c. of a serum such as mentioned in the experimental part would be necessary, and that the future of a serotherapy much depended upon the success attained in cultivating the organism in an artificial medium in large quantities. As a matter of fact we have been able to collect large quantities of comparatively pure organisms from each of the cultures of S. recurrentis, S. duttoni, S. novyi, S. gallinarum, etc., for various purposes (immunization, vaccinotherapy, etc.). In the serum of those who had just recovered from the relapsing fever a complement-fixation principle was demonstrated by Kolle and Schatiloff [248] and Korschum and Leibfried.[249] The reaction was said to be positive after the second attack.

In Weil's disease Inada and his co-workers found the presence

of a specific spironemalysin in the serum of convalescent man or guinea-pigs. The immune bodies develop after the second week of the disease and may be still present in individuals who had the attack more than four years previously. The Pfeiffer phenomenon is easily demonstrated by using the organ (liver or kidney) emulsions rich in the spironemata, or a culture and the immune serum in the peritoneal cavity of the guinea-pig. These investigators immunized goats and horses with the cultures of the causative agent (S. icterohæmorrhagiæ) for a period of more than a year and succeeded in producing a serum which prevents the infection against the lethal dose in guinea-pig in the amount of about 0.001 c.c. The clinical experience of this serotherapy which has now extended over many hundreds of cases proves to be highly encouraging.*

The question of immunity in syphilis is rather imperfectly understood. In human subjects it was once assumed that after the first infection complete immunity occurs, as evidenced by the extreme rarity of a reinfection. Later investigations seem to consider this assumption as incorrect, inasmuch as it was based upon the fact that the syphilitic individuals do not a second time contract a chancre or show a general skin eruption in spite of exposure to such an infection. This fact does not, however, necessarily denote immunity in the usual sense of the word. This state of refraction to the second infection is said to be due to the pre-existence of the same virus in the same individual who no longer reacts to the second inoculation with the original intensity or vigor, and the condition is designated by Neisser as "Anergie." At the same time Hutchinson showed the possibility in rare instances of an auto-inoculation, while Finger and Landsteiner [250, 251, 252] believe that a superinfection may take place in certain syphilitics. The effect of a superinfection may be a purely local manifestation or it may be subsequently followed by generalization; or it may again cause a general mobilization of the virus without a local manifestation. The character of the lesions produced by superinfection agrees with that of the lesions peculiar to different stages of the disease. If it occurs during the secondary stage the superinfected lesion will be a papule or other exuda-

* Personal communication soon to appear in print.

tive varieties, and if during the tertiary stage the result will be a gummatous product. This alteration of various tissues of a syphilitic individual in their reactivity to the syphilitic virus is designated as " Umstimmung " by Neisser, who regards this condition as a morbid state of the tissues brought about by the presence of Treponema pallidum. There once prevailed a vague impression that when cutaneous tissues are extensively involved there is less likelihood of the visceral organs being invaded by the syphilitic virus and *vice versa*,[253, 254] but there is not experimental proof to support this contention. Since the introduction of salvarsan and its derivatives in the treatment of syphilis, the instances of reinfection with typical or sometimes atypical chancres are not so rare, thus indicating that after a cure has been effected the human body reacts in the usual, or nearly usual,[255] manner. This also points to the absence in such cases of any lasting immunity after the first infection has been eradicated. A thorough investigation is required in order to ascertain whether or not a certain degree of immunity develops in some of the cured cases, thereby affording protection. In some ways the question of immunity in syphilis is comparable to that in protozoan diseases, in which, though latent, no typical infection can be reinduced until the first attack is completely cured, and where no congenital immunity has yet been demonstrated.

Let me now review the situation of the immunity question in experimental syphilis. Metschnikoff and Roux, Neisser and Bruck, and others found that monkeys which have once been infected with Treponema pallidum may prove refractory to subsequent inoculation. Metschnikoff[256] thought he succeeded in protecting a monkey against the infection by inoculating it with an attenuated living virus which was no longer able itself to produce typical reactions. That the vaccination against syphilis was not equivalent to that against variola in its fundamental principle was later demonstrated by Neisser and others, who were able to show that the monkeys which had been " vaccinated " with an attenuated virus and which were rendered "immune" to the subsequent inoculation with a fully virulent material were harboring the infection in various localities escaping the usual clinical

detections. Thus the emulsions, prepared from the bone marrow, spleen, etc., of the " vaccinated " animals were able to infect new susceptible animals. This phenomenon is similar to the state of anergy observed in syphilitic human subjects. Fontana,[257] Uhlenhuth and Weidanz,[258] Bertarelli,[259] Truffi [260, 261] and others pointed out that a rabbit which carries syphilitic keratitis in one eye is not refractory or immune to the infection in the other eye. A rabbit, one of whose testicles is infected with T. pallidum, offers no greater resistance in the other, which may be infected with the virus at any stage of orchitis preceding that on the opposite side. Tomasczewski [262] thought that a skin infection produced in rabbits in which scrotal lesions had been persisting for about two months was much milder than in normal animals. According to personal observations a rabbit in which a syphilitic orchitis, or keratitis, or scrotal chancre has been cured either spontaneously or through the administration of salvarsan, enjoys no perceptible immunity to syphilis. Truffi repeatedly inoculated rabbits with a fetal liver emulsion containing an abundance of T. pallidum, but found no immunity to develop. Uhlenhuth and Mulzer [189] immunized rabbits with the testicular pallidum emulsion without obtaining any decisive result, although in some cases they thought it exerted a beneficial influence upon the syphilitic process. In my personal experience it has been found that the susceptibility of the rabbit to syphilis is decidedly diminished in some animals by immunizing them with T. pallidum for several months. With a strain which gave 100 per cent. takes in normal rabbits' testicles only about 60 per cent. positive results were obtained in the immunized animals. This tends to show that the lower percentage of positive takes in the immunized rabbits may be due to the destructive influence of the treatment upon the invading pallida. But it was also found that in the immunized rabbits in which the inoculation succeeded the symptoms were not any milder. In fact, not only were the local reactions just as marked as in the control animals, but there was a tendency to the formation of generalized lesions. In two of the rabbits scrotal lesions developed after the intravenous inoculation of a virulent strain. It appears that an incomplete immunization exerts an adverse influence on the de-

fensive factors of the rabbit. This phenomenon finds verification in the work of Grouven and Sowade [263, 264] who recommended for the animal a few preliminary intravenous inoculations of the pallidum in order to insure a generalized infection through a subsequent intracardial introduction of the organisms in huge quantity. I also endeavored to ascertain whether a local administration of devitalized pallida (killed at 60° C.) on many successive occasions will not bring about a state of local immunity to Treponema pallidum, but my results were rather unsatisfactory, for the reason that the testicular parenchyma which was repeatedly inoculated with the pallidum emulsion underwent gradual atrophy and the resulting hard fibrous structure was no longer a suitable test-object for this fastidious parasite. Nevertheless I was able to produce small nodular lesions in two out of several rabbits so treated. Moreover, reinfection of the same tissues (cornea, testis, skin) after a spontaneous or chemotherapeutic healing has been found possible as long as the suitable structures of the tissues are preserved.

Our knowledge pertaining to the immunity phenomena *in vitro* is of more recent date, for the test-tube experiments with T. pallidum were made possible since the discovery of the organism and were particularly facilitated by the successful cultivation of the parasites on artificial media. Attempts to demonstrate the presence of a specific agglutinin for T. pallidum in the sera of human and experimental syphilis were made by Hoffmann and Prowazek,[265] Herxheimer and Löser,[266] Hoffmann[267] Brönnum and Ellerman,[268] Babes and Pinea,[269] Metschnikoff and Roux, Landsteiner and Mucha,[270] Zobolotny and Maslakowetz,[271] and others, with the pallida derived from the syphilitic tissues. Their experiments were indecisive, owing to the difficulty found in obtaining a pure material free from various tissue constituents. Uhlenhuth and Mulzer[189] found no agglutinins in the sera of the rabbit, goat and monkey after repeated intravenous injections of the rabbit's testicular emulsion rich in the pallidum. In 1910–1911, soon after obtaining pure cultures of T. pallidum, we started the immunization of rabbits with different strains of the organism. In the sera obtained from the immunized rabbits we were

able to demonstrate the presence of the specific agglutinins and complement binding principles for the cultivated pallidum strains. We were unable to produce with the sera any unmistakable agglutination of the pallidum derived directly from the syphilitic orchitis of the rabbit, but considered this to be due to the simultaneous presence of tissue debris and other cellular elements which may have interfered with the agglutination phenomenon. These sera were not strictly specific, but contained a small quantity of agglutinins for other treponemata obtained in pure cultures. There were also a sufficient number of specific complement-binding bodies, but there was at the same time a more or less definite group reaction for other treponemata. The work was continued later (1915–1916) by Akatsu at my laboratory with similar results. He was able to obtain a serum which could agglutinate the pallida in a dilution of 1 : 50,000.

In order to know whether syphilitic human sera have any definite agglutinating and complement-binding properties, a number of sera obtained from the various stages of syphilis were examined with pure cultures as well as with the tissue pallidum derived from rabbits' testicles. All experiments were unsatisfactory owing to the difficulty experienced in reading the reaction in the case of agglutination and also owing to the high anti-complementary powers of the antigens and the feebleness of the reaction in the case of the complement fixation test, except in the case of the pure culture antigens which fixed complement with the immune rabbits' as well as with some of the syphilitic human sera (chiefly late and tertiary cases). According to our experiments there is a certain degree of group reaction or the other treponemata (T. calligyrum, T. microdentium, T. mucosum, and S. refringens).

Kolmer [272] first described the agglutination of a pure culture of Treponema pallidum by the sera of rabbits injected with a living and heat-killed culture furnished by our laboratory. His results show that normal rabbit sera do not agglutinate the culture pallidum in dilutions as low as 1: 20, while the sera of immunized animals produced agglutination in dilutions as high as 1: 1280. No definite agglutination was observed with human syphilitic sera in

a dilution of 1:20 or higher. Nakano [273] also reported the presence of agglutinins in the sera of rabbits injected intravenously with a pure culture in dilutions from 1:10 to 1:70. Kissmeyer [274] immunized rabbits with a pure culture of T. pallidum and was able to obtain agglutinins in dilutions as high as 1:200,000 to 1:500,000 of the immune sera, while the sera from individuals with primary, secondary, tertiary and congenital syphilis contained agglutinins for the pallidum in dilutions of 1:100 and higher in a percentage of 40 to 60 out of 59 cases. Normal human sera may agglutinate the pallidum on dilutions as high as 1:50. Zinsser and Hopkins [275] state that normal rabbit serum may agglutinate the pallidum in dilutions lower than 1:10, but the sera of their immunized rabbits (intravenous injections of the pallidum cultures) agglutinated it in dilutions as high as 1:2000. They added that the normal as well as certain syphilitic human sera may agglutinate the culture pallidum in emulsions. Zinsser, Hopkins and McBurney [276] failed to observe any agglutination when the pallida from human lesions were mixed with the immune sera (rabbits and sheep) produced with the culture pallida. Zinsser and Hopkins demonstrated the treponemicidal bodies for T. pallidum (cultivated) in the immune serum produced by them.[277]

In the sera of animals experimentally infected with syphilis the presence of specific complement-binding antibodies for T. pallidum has not been satisfactory proved. It is true that we were able to demonstrate the positive complement fixation in the sera of animals immunized with cultivated treponemata, but this does not hold good when dealing with the syphilitic animal sera and the virulent pallidum strains found in tissues. On the other hand, these syphilitic sera do bind complement when mixed with pure cultures, not only of T. pallidum, but also of various bacteria, such as colon bacilli (Zinsser and Hopkins). Undoubtedly the phenomenon is non-specific but pathognomonic, as is the Wassermann reaction which is caused by certain lipoidal substances. These cultures must serve as the containers of the similar lipoids. Indeed, Craig and Nichols [278] long ago showed that the alcoholic extracts of the pure pallidum and pertenue cultures

produced almost equally strong complement fixation when mixed with the human syphilitic sera giving a positive Wassermann reaction with pure lipoidal antigens derived from other tissues. In a word, a syphilitic animal may give a positive complement fixation with various lipoids without at the same time containing any specific antibody for T. pallidum. In human syphilitic sera the same is also true, except in the sera of certain late and tertiary cases in which there may be a positive reaction due to the specific antigens and antibodies in the strict sense of Bordet-Gengou's phenomenon.[279]

The nature of the Wassermann reaction in the sera of human experimental syphilitic subjects is still unexplained, but one fact has been established *viz.*, that it is due to a peculiar change of the sera not specific for syphilis; it occurs in yaws, leprosy, trypanosomiasis, malaria (febrile period), and sometimes in malignant tumors. The fact that so many lipoidal substances as well as certain salts (sodium taurocholate, sodium cholate, etc.,) derived from different sources can bring about a positive fixation precludes any strict specific antigen-antibody reaction. According to personal observations, the lipotropic complement-fixation reaction is not present in immune rabbit sera which have been obtained by injecting the pallida repeatedly, and which contain a large number of specific complement fixation bodies from the pallidum strains employed for their production.

Closely related to immunity is the question of allergy in syphilis. From the chronic nature of the disease many investigators considered the possibility of its occurence at one stage or another. Jadassohn, Meierowsky, Ciuffo, Fontana, Neisser, Bruck and others made numerous observations which rendered the presence of allergy still more probable. These investigators were handicapped by not having a pure culture of T. pallidum. Soon after the isolation of the pallidum strains Professor Welch suggested that I undertake a study of this subject in human syphilis with the pure material. In the meanwhile it was ascertained experimentally that the prolonged treatment of rabbits with intravenous injections of the pure pallidum culture as well as with the organisms obtained direct from the rabbit's orchitis

lead to the production of a state of hypersensitiveness of the skin to the inoculation of the extract of a pure, heat-killed pallidum culture.[280] The reaction was found to be apparently specific for T. pallidum. There was no injurious effect following the injection into the rabbits of the heat-killed pallidum emulsion. The emulsion, since known as luetin, was employed as a means of diagnosing human syphilitic cases, with the result that the luetin reaction was found to be most frequently present in the latent tertiary and congenital syphilis cases where one would naturally expect most constantly to find the allergetic or hypersensitive state of the skin. As an auxiliary or supplementary factor in producing a positive luetin reaction I have already pointed out that the pathological state of the skin of chronic syphilitic patients designated by Neisser as "Umstimmung" a rôle in nearly 10 per cent. of tertiary cases in which the skin reacted intensively to the inoculation of the control emulsion without the pallida. No efforts were made to explain this peculiarity of hypersensitiveness of the skin of certain syphilitics. But a recent work of Camp [281] points out that the administration for many days of potassium iodide to a non-syphilitic individual produces in the skin a hypersensitiveness to any trauma, including the inoculation of the leutin. Probably this finding may furnish the solution of the problem of Neisser's " Umstimmung," or at least of one of the contributing factors. The clinical evidences thus far accumulated seem to show, however, that in a large number of cases the luetin reaction was positive in spite the fact that no iodide had been given during the period when the test was applied. Recently Akatsu [282] at my laboratory carried out several series of experiments regarding the influence of potassium iodide upon the reactivity of the skin of rabbits to the intradermal inoculation of the luetin, control fluid and plain bouillon. The iodide was administered intravenously for a period of from 7 to 9 days, given in increasing doses of 0.5 to 2 c.c. of a 10 per cent. aqueous solution. At the end of seven days or later the skin was tested for the luetin, control and plain bouillon. It was found that the skin of normal rabbits did not react to the injections after the iodide treatment. There was no change in its reaction to the trauma. The skin of

the rabbits which had been previously rendered hypersensitive to the luetin by means of prolonged immunization with pure pallidum cultures mostly remained the same, that is, it reacted to the luetin with the same intensity as it did before the administration of potassium iodide. Only in a few instances was the reaction somewhat intensified. There was no definite reaction to the control emulsion of plain bouillon. In some rabbits in which the testicular orchitis after several months had shrunk to a small fibrous nodule the luetin reaction was mildly positive, but the intensity of the reaction was but little influenced after the injection of potassium iodide, except in a few rabbits where the second tests came out more distinctly. The above findings show that the potassium iodide has no noticeable influence upon the reactivity of the skin of normal as well as of syphilitic rabbits. It would be interesting to study whether in other spironematoses (relapsing fever, tick fever, rat-bite disease, infectious jaundice) there appears any skin allergy comparable to that described for other bacterial infections (typhoid, gonorrhœa, etc.). In cases of yaws the skin reacts to the intradermal inoculations of the luetin and of the frambœsin with equal intensity and cannot be differentiated by this method (Baermann and Heinemann) [283]

The last and probably the most important field is chemotherapy. The inauguration of modern chemotherapy by Ehrlich is as interesting as it is romantic. It can be traced back to Schaudinn's suggestive but unsupported theory that the spirochætes represent a stage of the life-cycle of trypanosomes, or at least were closely related to the latter. The introduction of organic compounds of arsenic into the treatment of trypanosomiasis was promising much when Schaudinn discovered T. pallidum which he regarded as a protozoa allied to the trypanosomes. Ehrlich took up experimental chemotherapy in connection not only with the latter, but also with the newly discovered spirilloses, as he called them, including syphilis and the fowl fever caused by S. gallinarum. The achievements of Ehrlich and his collaborator Hata, in discovering salvarsan for the treatment of these two diseases, mark a new era in modern chemotherapy. To review this phase of the spirochæte problem would be out of the scope of

my present paper. Suffice it to say that to the great pioneers, Schaudinn and Ehrlich, Metschnikoff and Neisser, we owe an inestimable debt, not merely for their own researches, but also for rekindling in us the sublime stimuli which have already inspired so many investigators to discover new facts, and which will continue to urge us still more to take up this task and to extend our knowledge regarding the classification, morphology, biology, pathogenesis, and experimental as well as clinical aspects of the micro-organisms known as spirochætes.

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THE SUSCEPTIBILITY OF MAN TO FOREIGN PROTEINS *

PROF. WARFIELD T. LONGCOPE, M. D.
Columbia University and the Medical Clinic of the Presbyterian Hospital, New York

THROUGHOUT the literature of the last century one may find isolated statements regarding the unexpected effects upon certain individuals of substances now recognized as containing proteins, and perhaps the most significant of these are the observations of Blackley in 1873, on the effect of grass pollens upon the nasal mucous membrane, conjunctivæ and the scarified skin of individuals sufferings from hay fever. About 1869 and 1870 when transfusions of sheep's blood were much in vogue, it was frequently noted that an urticaria appeared as a late effect of this treatment. It was not, however, until the introduction of diphtheria antitoxin in 1894 that the results of injections of foreign proteins in man received serious study. Lubinsky [1] in the year 1894 described what is now the perfectly familiar picture of serum disease as a sequel to the injection of diphtheria antitoxin, but not until the careful investigations of von Pirquet and Schick [2] in 1905, was any important light thrown upon the process. They immediately recognized the importance which the so-called antibodies against foreign protein, in this instance horse serum, might have in the production of serum disease. And the subsequent observations and experiments by Otto, Rosenau and Anderson, Gay and Southard, Friedmann, Doerr, Friedberger and a host of others [3] have made it quite clear that the introduction of native foreign protein follows essentially the same biological and chemical physical principles in man as in the lower animals.

It is only necessary to point out here that the introduction of the long series of foreign proteins that have been subjected to experiment is followed by the production in the animal body of various antibodies which have the power to unite with the

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original substance or antigen to produce a new effect. This new effect may be obvious and best studied by mixing antibody and antigen outside the body, as is the case with the hæmolysis, the bacterial agglutinins, bacteriolysins and precipitins. It may be obtained both *in vitro* and *in vivo,* as is the case with antitoxin, or it may be demonstrable almost exclusively in the animal body, as is the case with anaphylaxis.

The bare outlines of the experiments from which our knowledge of anaphylaxis has been derived may be summed up in a statement of the following condition: An animal is injected subcutaneously, intraperitoneally, or intravenously with a small amount of foreign protein, for example horse serum. This produces no visible effect. If, however, after an interval of at least nine to fourteen days, a second injection of the same protein is made, the animal becomes ill immediately, presenting characteristic symptoms which differ only slightly for different species, while if the dose of serum is properly gauged and made into the peritoneum or vein the animal dies within five minutes to one hour. This is known as active anaphylaxis. A second condition known as passive anaphylaxis was first described by Gay and Southard,[4] and also by Otto and Friedmann,[5] who showed that the blood of an animal sensitized to horse serum confers this specific sensitiveness to a normal animal after a period of fifteen to eighteen hours following the inoculation of the serum from the sensitized guinea-pig, and lasts a few days, or at most weeks. It is dependent upon the presence in the blood of the actively sensitized animal of antibodies for the specific proteins which are thus transferred passively to the normal guinea-pig. This transfer may take place during the pregnancy from mother to offspring. Third, the state termed anti-anaphylaxis by Besredka and Steinhardt,[7] in which for a short period following the anaphylactic shock, the actively sensitized animal becomes insensitive to injections of horse-serum. Following this period of anti-anaphylaxis an animal may again become sensitive to the specific protein and remain so for months or years. Finally, it was shown by Rosenau and Anderson[8] that the repeated injection of horse serum at intervals of two to three days, pro-

duced a refractory condition towards subsequent injections of the specific protein, so that for a long period of time they no longer react to such an injection with anaphylactic symptoms. This refractory state, however, finally gives way to sensitiveness and eventually the animal is again susceptible. During the refractory state which has been called one of immunity, antibodies for horse serum may be present in great concentration in serum, and when injected into the normal guinea-pigs will passively sensitize them to the protein.

After this brief statement of the fundamental principles of anaphylaxis, it is possible to consider in more detail the process as it develops in man, for the introduction of antitoxic and antibacterial sera has given ample opportunity for an exact analysis of the effect of the single and repeated injections of foreign protein. This, as a rule, has been horse serum, though the serum from any foreign species will give the same results. As in animals, so in man, primarily injections of horse serum given by any route produce no immediate symptoms. In the vast majority of instances it remains, as can be shown by the presence of precipitinogen and by anaphylactic methods, in the circulation for long periods of time and is excreted very slowly, if at all, by the kidneys. For a period of 6 to 10 days following primary injection, known as the incubation period, there is nothing to be observed, but at any moment after the sixth day, typical serum sickness may appear.

The symptoms and signs of this disease are as striking and characteristic as any natural disease process with which we are familiar. Usually a skin eruption with intense itching preceded by glandular enlargement ushers in the attack. The eruption starts as a rule at the point of inoculation but spreads rapidly over the entire body. Urticaria is most common, but there may be a patchy or diffuse erythema, scarlatiniform rashes or multiform eruption. Œdema of the face and ankles is usual and may effect the entire body. In rare instances it is localized to the pharynx or larynx, and in one instance a transient hæmiplegia was supposed to be caused by local œdema of the meninges. The temperature is elevated and there is general malaise and

headache with prostration, and rarely nausea or vomiting. Joint pains are very common in severe cases. They are always multiple, and though the pain is exquisite on motion, there is little tenderness and no swelling or reddening. Quite regularly the lymph-nodes are enlarged; they are often swollen to the size of walnuts in the region draining the primary injection and may be excessively tender. The spleen is sometimes enlarged; in five to nine per cent. of the cases there is albuminuria and, as I shall point out later, there are often disturbances in the functional activity of the kidneys. Examination of the blood, according to von Pirquet, shows a primary polymorphonuclear leucocytosis with subsequent leukopænia and absolute increase in the lymphocytes. The disease may last for 24 hours to 20 days or longer. In the severest cases there are one to four relapses, which usually occur after the use of large amounts of serum, and come, according to Axenow,[9] at ten-day intervals.

The occurrence, intensity and duration of the disease depend upon several factors: First, upon the amount of serum used. The disease is not usual after small doses, but is extremely common after large ones. The statistics of Weaver[10] based on a study of 801 reported cases show an incidence of 10 per cent. after the injection of 1 to 10 c.c. of serum. Most of the attacks are mild, but the incidence increases in almost direct proportion to the amount of serum used, until, when quantities over 90 c.c. are employed, 75 to 100 per cent. of the patients develop serum disease. Secondly, it seems possible, from the statistics of Bokay[11] and Schulz,[12] that the incidence may depend to some extent upon the source of the serum. Apparently, certain horses yield a serum which is much more likely to be attended by serum disease than others. And, finally, the individual characteristics of the patient play a rôle, for the same dose of serum from the same source may produce quite different effects in different persons.

A second injection of serum before the tenth day, or before the onset of serum disease, is not attended by immediate symptoms. It has already been observed that in the case of animals repeated injection of horse serum at 2 to 3 days' intervals pro-

duce a condition which makes them refractory to subsequent injections, which state may last many weeks. By the same method in human beings the incubation period for serum sickness may be prolonged for weeks, but its final appearance cannot always be prevented, and after these multiple doses at short intervals the attack, when it appears, is, according to Goodall,[13] especially severe. If, however, a second injection is made after the 10th day, von Pirquet and Schick have shown that the following reactions may occur: (1) The local immediate reaction. Within fifteen minutes to an hour after subcutaneous injection of serum, œdema, erythema or urticaria appears at the site of inoculation. (2) The general immediate reaction which is characterized by a more or less severe general reaction coming on within 12 to 24 hours after the inoculation and having the characteristics of a fulminant case of serum sickness. The most violent and general immediate reactions are accompanied by such symptoms as dyspnœa of asthmatic type, cyanosis, collapse, nausea and vomiting and suppression of urine; symptoms which are not regularly seen in the ordinary form of the disease. (3) The accelerated reaction or form in which the incubation of the serum sickness falls between the immediate reaction and the ordinary reaction and comes on within a period of 3 to 5 days after inoculation, and finally the second injection may be followed simply by the normal form of serum sickness. Any of these reactions may occur separately or may be combined in one and the same patient.

The actual occurrence of the immediate general reaction following a second injection varies according to different observers. It is noted most often, according to von Pirquet and Goodall, between the 35th and 80th days after the primary inoculation of serum, and occurs in about 60 per cent. of the cases when serum is injected in large quantities at this period. An injection made after three months is usually followed by an accelerated reaction and when years have elapsed the general reaction may be of the ordinary type. The immediate reaction, though it may be severe, has, as far as I know and as the statistics of Park[14] and Nemmser[15] and Cuno[16] show, never caused death when the inoculation is made subcutaneously. Following a second intra-

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of pure serum and of serum in 1: 10 and 1: 100 dilutions has been employed. After these minute doses skin sensitiveness does not appear until serum disease has set in, but once having made its appearance the reaction increases in intensity and has been present by the 15th to 20th day in most instances. A reaction of equal intensity has been obtained in several adults two to three years after an injection of antitoxin. The intradermal method is much the most sensitive of any of the cutaneous tests and may be strikingly positive with serum in 1: 100 or 1: 500 dilution, although the scarification method such as used for tuberculin by von Pirquet gives a negative response with undiluted serum. It is, as far as we have observed, specific, and cannot be brought about in these cases by the use of various other animal sera, egg-white, casein or vegetable proteins. It consists in the appearance after a period of ten to fifteen minutes of an elevated yellowish firm wheal, surrounded by a bright red erythematous areola. This increases rapidly in size until the wheal reaches at the end of 30 to 45 minutes, 1–2 cm. in diameter, and the erythematous zone 3–6 cm. in diameter. During this period the patient often complains of itching at the site of inoculation. At the end of an hour the reaction fades and in twelve hours nothing is to be seen. Salt solution, human sera and various other animal sera result in the formation of a minute white papule 5–6 mm. in diameter, about which there may appear a narrow red zone. This traumatic reaction fades in 5 to 15 minutes and is almost invisible at the end of half an hour, when the true reaction is at its height.

Knox, Moss and Brown [27] have found that under similar conditions a skin reaction is obtained in the rabbit. It makes its appearance first on the 11th day after primary inoculation of serum and is present in most cases by the 16th to 22nd day. It differs from the reaction in the human being in that the development is much slower, attaining its height only after 12 to 24 hours, while it persists much longer, lasting two to three days. The skin reaction must be considered as a delicate and probably specific reaction of an individual artificially sensitized to a

foreign protein and makes its appearance from 5 to 15 days after the primary injection.

At about the time that this sensitiveness appears and sometimes immediately before the onset of the serum disease, other reactions occur which are considered evidence of the formation of antibodies to the foreign protein. These are represented by the appearance in the blood serum of precipitins for the foreign protein and the substance which is capable of transmitting the sensitive state passively from one animal to the other. Hamburger and Moro,[28] who first demonstrated precipitins for horse serum in the blood of cases of serum disease, and Doerr and Russ,[29] as well as Weil,[30] experimenting later in animals, have attached much more importance to the precipitins as a part of the anaphylactic reaction, though von Pirquet was inclined to regard the production of precipitins as an accompanying phenomenon, rather than as one upon which anaphylaxis is dependent. Wells [31] has followed the curve of production of these antibodies during serum sickness in children receiving diphtheria antitoxin. In some cases precipitins are demonstrable about the sixth day after injection of serum and before the sickness starts, but with the onset of serum sickness the precipitins diminish or disappear to increase rapidly after the disease, to a high degree of concentration. A diminution again takes place 16 days later and finally the precipitins can no longer be demonstrated.

More closely related to the anaphylactic state than the precipitin, is the presence of the substance which is capable of causing passive sensitization, variously designated immune substance, anaphylactic antibody, anaphylactin or allergin. Though Doerr and Russ [32] have undertaken to measure quantitatively the amount of this antibody in a given specimen of serum, there are certain factors, as has recently been shown by Lewis,[33] which complicate such a quantitative analysis of the anaphylactic antibody, and consequently the result obtained from passive transfer can only roughly measure the actual amount of this substance in the actively sensitized animals. That there is some parallelism between the amount of precipitin and quantity of anaphylactin has been shown by Burkhardt,[34] but when several injections of

serum are given to sensitize actively the two reactions do not run parallel. Weill, Hallé and Lémaire [35] found that the blood of rabbits receiving a single injection of horse serum showed the power to transmit the anaphylactic antibodies to guinea-pigs, after the lapse of ten days. This increased in intensity up to the 25th day and gradually disappeared after the 60th day. When, however, repeated injections of horse serum were employed at two months' intervals, the ability of the rabbits' serum to sensitize guinea-pigs appears immediately after the last injection but does not persist as long. Anderson and Frost [36] found that the blood of guinea-pigs contained "anaphylactin" for as many as 450 days after sensitization, and as Rosenau and Anderson originally noted, guinea-pigs made refractory to anaphylactic shock by repeated injections of horse serum, still carry in their serum anaphylactic antibodies in large amounts. Attempts to demonstrate this phenomenon in man have met often with indifferent success. Anderson and Frost,[37] as well as Archard and Flands,[38] and Weil [39] and Yamanuchi,[40] report isolated successful results, but Novotny and Schick [41] obtained positive results in only two out of twelve cases. Grysez and Bernhard,[42] however, were more successful and with the blood drawn from eleven children 5 to 234 days after injection of diphtheria antitoxin, were able to sensitize guinea-pigs passively to horse serum. Dr. Rackemann and I have made some observations upon the appearance of this anaphylactic antibody in ten individuals receiving horse serum. We have found that the anaphylactin was present in one or two instances before the appearance of serum disease, but in the vast majority of cases it is only demonstrable at the subsidence of the symptoms. Once having appeared at this time, however, it may persist for many days and be present in such concentration that one can readily transfer the sensitiveness from man to a guinea-pig.

It will thus be seen that toward the end of the incubation period of serum disease, certain antibodies and immune reactions, namely, the skin reaction, the precipitin reaction and anaphylactin, can occasionally be demonstrated in the blood serum. With the appearance of the symptoms they diminish or disap-

pear to reappear with great intensity at the subsidence of the attack. Since the serum or antigen during this entire period may still be demonstrated in the circulation, it is highly probable, as was proposed by von Pirquet, that serum disease is brought about by a union of the rapidly forming antibodies and the circulating antigen. The theoretical explanation as to the place and cause of this union has been the subject of much discussion. The union, of course, may take place in the circulating blood, thus liberating toxic substances that produce the symptoms of serum disease or anaphylaxis; second, the union may occur in the tissues and cells of the body; or, third, it may proceed in both situations. Friedberger, who has experimented extensively along this line, has advanced first one and then the other view, and in the human being the appearance of precipitins and the anaphylactic antibodies in the blood stream before the onset of serum disease, might lead one at first to suppose that the reaction was principally in the circulating blood, as was the original idea of von Pirquet. The observations of Pearce and Eisenbrey,[43] of Schulz[44] and those of Dale,[45] which have been extensively amplified and confirmed by Weil,[46] show definitely that the cells of sensitized animals and certainly the smooth muscle of the uterus, freed from all traces of the body fluids, are capable of reacting violently when brought in contact with the specific antigen toward which the animal is sensitive. The original observation of Rosenau and Anderson, repeatedly confirmed, that the blood serum of an animal made highly refractory by repeated injections of horse serum, may still contain large amounts of anaphylactic antibody, is in support of the idea at present quite widely accepted that the presence of these antibodies in the serum is a source of protection rather than harm. Circulating in the blood in large quantities they unite slowly with the reinjected antigen, or at least "fix" it so that the antigen as such is prevented from coming in contact with the cells of the body. The facts which have been brought out in the study of serum disease in man might be interpreted in the light of either theory.

The onset of serum disease is probably, therefore, a visible evidence of the development of general sensitiveness and repre-

sents a more or less violent reaction between the circulating antigen and antibody which is in process of development, certainly in the cells, but possibly also in the circulating blood. It is followed by a rapid expulsion of antibodies into the circulation, and shortly afterwards by a period of hypersensitiveness at which time the reinjection of serum may call forth a violent general reaction. Subsequently, this period of hypersensitiveness diminishes and antibodies may disappear from the circulation. The injection of serum at this time, owing to the slight concentration of antibodies, does not produce an immediate general effect, but excessive antibody formation under these circumstances, as von Pirquet and von Dungem have shown in the case of precipitins, is much more rapid than in the normal individual and the general reaction, or accelerated serum sickness, appears. Finally, with a complete loss of sensitiveness the individual returns to the normal state and the reaction is of the normal type.

There is not time to discuss the question as to whether the union of antibody and antigen is of chemical nature, whether it depends upon ferment action, or whether, as is the view that Zinsser [47] has brought forward, some changes in the physical properties of the serum determines the union of these two substances. To elucidate the actual nature of the toxic substance that is formed by this union, much thought and experimentation have been expended. It is only a step from the early observations of Pfeiffer,[48] and Wolff-Eisner [49] upon the poisonous products of bacterial disintegration to the work of de Waele,[50] Biedl and Kraus,[51] Vaughan,[52] Friedberger,[53] Weichardt,[54] Schittenhelm, and many others who have shown that the products and by-products of protein digestion when injected into various animals may cause symptoms almost identical with true anaphylactic shock. These observations soon suggested that with the union of antibody and antigen, the antigen or foreign protein is digested to form poisonous split-products. To confirm this idea evidence has been brought forward by Abderhalden,[55] Ammerer, and Pincussohn,[56] by Pfeiffer and Jarish,[57] Zunz and Gyärgy,[58] and by Jobling and Petersen,[59] to show that during anaphylactic shock in dogs products of protein digestion appear and that the non-

protein nitrogen, together with the proteose of the serum, are distinctly increased.

So far we have discussed only artificial sensitization in man, but it is now necessary to direct attention to a state in which sensitiveness to foreign proteins exists without the known introduction of these proteins. It has been repeatedly observed that the first injection of any foreign serum may be attended in man by a violent immediate reaction. One of the earliest instances to come into prominent notice was the immediate death of Professor Langerhans's young son after receiving for the first time an injection of diphtheria antitoxin. Gottstein [60] reported a few such cases in 1896, and in 1909 Gillette [61] collected from the literature 30 in which sudden collapse or death followed immediately upon the first injection of antitoxin. It is important to note that 22 of these cases gave a history of asthma or of some respiratory disease. The patient immediately after the injection shows great uneasiness, perhaps complains of constriction in the chest, has violent dyspnœa, the face swells and becomes purple, he falls in collapse and may be dead in five to ten minutes. In attacks which are somewhat less fulminant there is violent asthma, cyanosis, collapse and a giant urticarial eruption. These perhaps are the most striking and certainly terrific demonstrations of spontaneous sensitiveness to foreign protein, but they only represent one of many such sensitizations which have been long recognized as idiosyncrasies. The early work in 1873 of Blackley [62] upon hay fever, I have already mentioned. It is of considerable historical interest to note that he associated this condition as early as 1853 with the inhalation of pollen grains, and showed definitely that the instillation of certain pollens in the nose and eye produced in hay fever patients œdema and congestion of the mucous membrane with lacrimation. He also made the important observation that pollen grains rubbed into a scarification of the skin called forth an intense itching and local œdema. The work of Dunbar [63] in 1903 and 1904 showed that this was a specific reaction and later Weichardt [64] brought out the fact that it was due to the protein of the pollen. From this it was only a step for Wolff-Eisner [65] in 1906 to suggest that hay fever had its origin

in sensitization to proteins of various pollens and later to apply this principle to the explanation of the origin of the urticarias succeeding the ingestion of certain foods.[66]

So common, indeed, is this conception of protein sensitization that attempts have been made to explain thereby all physiological and pathological processes from childbirth to epilepsy. But in spite of these unwarrantable views, it is certainly an important factor in some pathological processes and for this reason it is highly essential that we should understand accurately what true protein sensitization means and analyze the hypotheses that may be evolved from this fascinating but very obscure subject.

A survey of the literature brings forth many interesting examples of what seem to be true spontaneous susceptibility to various foreign proteins. The symptoms produced from contact with these proteins are usually referred to the respiratory tract, the gastro-intestinal tract and the skin. Hay fever is the example of protein sensitization most thoroughly studied, in which the symptoms brought on by contact with foreign proteins are usually confined to the respiratory tract and the conjunctivæ. In a certain proportion of cases of asthma the attack simulates, as the fundamental experiments of Auer and Lewis,[67] suggested to Meltzer,[68] mild anaphylactic shock in the guinea-pig and as has been demonstrated since, it may be precipitated by inhalation, subcutaneous injection or ingestion of foreign proteins to which the individual is sensitized. The most striking examples are to be found in the patients whose attacks invariably occur when they come in close contact or even in the near vicinity of such animals as horses, dogs, cats, rabbits or mice; or in patients who develop from their hay fever true paroxysms of asthma. The subcutaneous injection of even the minutest quantities of solutions of these proteins will precipitate violent paroxysms of asthma, and it is in such spontaneously sensitized individuals that the first injection of diphtheria antitoxin has produced violent symptoms or death.

The gastro-intestinal disturbances dependent upon sensitization to eggs, which is not uncommon in children and may occur in adults, has been studied, especially by Schloss,[69] Lesné [70] and

Richet, Fils and Talbot.[71] Sensitization to cow's milk as recently pointed out by Kleinschmidt [72] is another cause of gastro-intestinal disturbances in children. H. R. Smith [73] first drew attention to the importance of sensitization in a case of buckwheat poisoning, and similar symptoms after eating such foods as strawberries, melons or other fruits or vegetables, certain form of meats and shellfish, are widely recognized and are familiar as idiosyncrasies.

Various skin eruptions are associated with direct or indirect contact with foreign protein. In some instances an urticarial eruption always follows contact with the fur of one or another animal or the juice of plants. Wechselmann [74] has described a form of dermatitis which comes on in satinwood workers ten to fourteen days after they start their work. Talbot and Lesné and Richet have especially called attention to the eczema which so frequently is seen in children sensitive to egg-white and milk. An increased susceptibility, which Goldschmidt [75] has described to the irritating odor and juices coming from the living ascaris, is probably to be classed with these cases.

A few instances of hypersensitiveness to the sting of insects are described. I know of one individual in whom the sting of the mosquito produces enormous areas of œdema, similar to the so-called angioneurotic form, and in another case a sting of a wasp was followed by symptoms exactly like the immediate reaction in serum disease. Great local swelling with erythema and œdema, collapse, general urticaria; joint pains appeared immediately on two occasions after this individual was stung. Finally, there is a very definite group of cases of which I have seen three or four, in whom suddenly without assignable cause, the patient is stricken with an illness which is precisely like serum disease and which may recur at the end of several months. At the onset there may or may not be nausea and vomiting, but always there is a general urticaria associated with slight fever, swollen tender lymph-nodes, arthralgia and usually a mild albuminuria. Indeed, some of the attacks of Henoch's purpura are not unlike serum disease and we have found at least one case sensitive to several foreign proteins. Even more obscure pathological processes, such

as eclampsia, have been explained upon the basis of protein sensitization, in this instance to the chorion or fetal tissues, but the evidence for such assumptions at the present time does not admit of further discussion of these very obscure conditions.

Reference must be made here to the interesting drug susceptibilities which apparently may be spontaneous or acquired after the repeated subcutaneous or intravenous use of certain chemicals. In this connection reactions to the iodine and arsenic compounds have been most carefully studied. Stäubli [76] and Kaufman [77] have called attention to the increasing local inflammation which patients are likely to experience after repeated injections of cacodylate salts. With the introduction of salvarsan many observers have noticed anaphylactic-like symptoms during the intravenous administration of this drug. Iwaschenzoff [78] has reported many such instances and Draper,[79] who has observed it in about 55 per cent. of all treated cases, states that it is never noted at the first injection, rarely at the second, and that if it occurs at all, it usually does so at or after the fourth. The patient complains of oppression in the chest, is restless, has dyspnœa, shows suffused face and conjunctivæ and cyanosis. Afterwards there may be vomiting and occasionally a blotchy urticaria appears.

Up to the present time it is not known that antibodies are produced by any substances other than those which contain a high protein molecule, and at first the explanation of these reactions was difficult. Friedberger and Ito [80] have called attention to the fact, however, that the serum of a guinea-pig may be so altered by treatment with the tincture of iodine that a mixture of the two will sensitize the guinea-pig to subsequent injections of the same mixture of its own serum and iodine or sodium iodine or to Lugol's solution. A first injection of Lugol's solution sensitizes to this mixture of serum and iodine but not to potassium iodine or to Lugol's solution alone. Swift [81] has been able to obtain essentially the same results with salvarsan, and the explanation given by these observers for the idiosyncrasies to iodides and the anaphylactic-like symptoms after the intravenous injection of salvarsan is that the combination of the drug with the patient's serum forms a new protein compound to which he may be actively

sensitive. The reports by Bruck [82] and Klaussner [83] of passive sensitization with the serum of patients showing drug idiosyncrasies have been disproved by Züeler [84] and Pöhlmann.[85]

These examples—and they might be multiplied many times—represent a group of morbid conditions which have received most study up to the present time. A careful comparison of the symptoms following an injection of foreign protein in the sensitized man with those seen in various animals during anaphylactic shock, shows a close similarity. The multiplicity of symptoms is greater in man than in any other one animal. But even typical serum disease has been observed in calves by Beclère, Chambon and Menard,[86] and in rabbits we have noticed enlargement of the lymph-nodes with subcutaneous œdema from the sixth to the tenth day after intravenous injection of large quantities of horse serum.

Besides this similarity between the symptoms in man and animals, there is a close analogy between the development of specific immune bodies in the two species. This was especially emphasized in considering serum disease and the same type of immune bodies has been shown to exist in the spontaneously sensitized individual.

Precipitins to cow's milk have been found in the serum of nurslings by Kleinschmidt, and Clowes [87] and Koessler [88] have noted precipitins for pollen in the serum of hay fever patients. Bruck [89] was able to sensitize guinea-pigs passively to pig serum with the blood of a patient who developed violent gastro-intestinal spasm and urticaria after eating pork. Similar results were obtained by Schloss [90] with the serum of his patient who was sensitive to egg-white and by Koessler with the serum from cases of hay fever.

The specific skin sensitiveness which is obtained in immune rabbits and guinea-pigs and which is a characteristic of the artificial sensitization by antitoxic sera in the human being is an especially prominent feature of the spontaneously sensitized.

The application of minute quantities of specific protein to the skin of such a patient causes a severe local urticaria and erythema and just as the skin reaction has been employed to estimate the degree and persistence of sensitization in people subjected to one

or two injections of antitoxic sera, so it has been used now quite extensively by Schloss, Talbot, Koessler, Cooke,[91] Goodale [92] and others, to determine the protein to which these people may be sensitive. There can be no doubt that the skin sensitiveness is solely dependent upon the introduction of protein, for Schloss obtained cutaneous reactions in his case of egg idiosyncrasy with pure ovamucin, ovoglobulin, ova-albumin and ovomucoid. We, too, have obtained striking skin reactions with chemically pure phaseolin, prepared from beans and kindly sent us by Professors Mendel and Osborne of Yale University.

An analysis of the conditions of sensitiveness in these patients, however, shows that they differ in some respects from the artificially sensitized. In the first place, the degree of hypersusceptibility is generally much greater than is ever reported in the artificially sensitized. In the second place, the sensitization is usually multiple, and in the third place, the method of sensitization is problematical.

Not only is the increased susceptibility seen in the general violent reactions following subcutaneous and intravenous injections of the specific proteins which may be so great that a subcutaneous injection of 1 c.c. of 1 : 1000 dilution of serum, as stated by Koessler, or 1 c.c. of a 1 : 50,000,000 dilution of pollen extract, will bring about general urticaria and asthma, but it is also noticeable in the response of the skin and mucous surfaces. Patients artificially sensitized with horse serum rarely develop horse asthma, though such a thing is possible, for Sewall [93] reports an attack of rabbit asthma in a man who had been injected with rabbit serum. Mere contact with horses will not produce anaphylactic symptoms in guinea-pigs sensitized to horse serum, though instillation of serum into the nose or trachea will do so. Even rarer are gastro-intestinal symptoms following ingestion of proteins to which either man or animal has been actively and artificially sensitized. In the sensitized guinea-pig numerous observers have attempted to produce shock by way of the gastro-intestinal tract, but all have met with complete failure. We have seen, on the other hand, that gastro-intestinal symptoms are common in the individual with spontaneous sensitization. The relative susceptibility of

the skin and mucous membranes in these individuals may, however, vary greatly, and though the inhalation or intradermic injection of foreign protein may bring about general urticaria and asthma, ingestion of the same protein may be absolutely harmless and without the slightest effect upon the gastro-intestinal mucosa. Thus, one of our asthmatics whose skin was highly sensitive to sheep serum and who on several occasions developed urticaria and asthma after a minute subcutaneous injection, could take by mouth large quantities of sheep serum without the slightest gastro-intestinal symptoms.

The second point of difference, namely the multiple sensitiveness, is very difficult to interpret. Whereas the artificial sensitization of both man and animals is within limits specific, these patients frequently give a history not only of idiosyncrasies to more than one substance, but as most observers who have studied their skin reactions have noted, give positive skin tests to several different proteins. In the patients whom Dr. Rackemann and I have investigated this has been quite regularly noted. We have found, however, that the skin reaction and occasionally the general reaction is limited to certain groups of proteins. Patients can thus be roughly classified as those who react to the sera of animals, those who react to eggs or the sera of fowls, those that react to the extracts of shellfish and those that react to the protein of plants. Occasionally the same individual may show sensitization to two or three groups, but as a rule it is confined to one. These reactions, too, we have found are not limited solely to abnormal patients, since in a long series of apparently healthy individuals, we have found two or three who show skin sensitiveness to two or more foreign proteins.

It is, of course, possible, as was shown early by Rosenau and Anderson, to sensitize the guinea-pig to at least three different proteins to each one of which the animal will react specifically, and one might suppose the same could easily happen to human beings once the path to sensitization was open. Besides this it is known that only within certain limits is the anaphylactic reaction specific in as much as guinea-pigs highly immune to the serum of one animal will show mild anaphylactic symptoms when

injected with the sera of a closely allied species. This is especially true for man and ape, horse and ass, while Wells and Osborne [94] have shown that the same is true of plant proteins, for an inter-anaphylactic reaction takes place between chemically pure gliadin from wheat and hordein from barley.

The wide range of sensitiveness, however, and the great unlikelihood that the person might come in contact with many of these proteins, makes it very doubtful whether this, which is accepted by some as an explanation, is correct.

A second possibility, namely, that the sensitization is non-specific, must be considered. It is known that when native proteins are heated or brought into contact with alkalies or other chemical agents, their physical properties are injured and they no longer give specific biological reactions. Reference has already been made to the effect that iodin and salvarsan may have. The protein under these circumstances is said to be denatured. By this process of denaturizing, Landsteiner and Prasek [95] were able to destroy partially the specific precipitin reaction to horse serum. It is possible to conceive, therefore, that during a natural process of sensitization, through whatever route, the native protein is denatured, and when it comes in contact with the cells and tissue juices, produces a non-specific sensitization. This might be especially true if the protein enters the body by way of the gastro-intestinal tract, and is accepted as the explanation by Otto and Hoefer.[96]

Although the mode of sensitization might seem at first glance easy to explain, there are certain factors involved that really make its interpretation very difficult. The paths open for such a sensitization are, of course, numerous. Wounds of the skin and mucous membranes might readily be held responsible and even through the unbroken mucous membrane sensitization may be accomplished in animals. The history of many of these cases of spontaneous sensitization suggests that the process has taken place by one of these routes. But such an explanation is not applicable to all cases, and cannot account for the fact that an infant shows violent symptoms the first time it is fed an egg.

The history of idiosyncrasies in certain families, such as the

tendency to asthma or hay fever, or the susceptibility to certain foods, has long been recognized as very common. Cooke obtained a history of some idiosyncrasies in the immediate families of 129, or 63.8 per cent., of 205 cases of hay fever and every one who has written on the subject of asthma draws attention to the familial tendency. Lesné and Richet report the occurrence of egg sensitiveness through three generations. Inheritance must, therefore, be considered as a possibility. Immunity to certain poisons may be transmitted from mother to offspring, and it has been definitely shown by Rosenau and Anderson,[97] Gay and Southard [98] and by Lewis,[99] that the same is true of hypersensitiveness to horse serum in guinea-pigs. Hypothetically, this may take place in one of three different ways: (1) As true inheritance through the germ plasm of the cells, either of the father or mother; (2) by direct influence of the immunizing agent that affects the mother, upon the cells of the fœtus which would produce active immunity in the child; (3) by passive transference of the immune bodies from mother to fœtus by way of the blood or milk.

It was shown by Ehrlich,[100] in studying the transmission of abrin and ricin immunity in mice, that this is conferred only by the mother and that even when the mother is immunized during pregnancy, the immunity in the offspring lasts but a few weeks. He concluded that this was, therefore, in no sense of the word a true inheritance, but the passive transference to the fœtus or child of immunity through the blood or milk of the mother. The immunity is not transmitted to the second generation. The same principle has been established in the transference of other forms of immunity and in experimental anaphylaxis. Groer and Kassovitz [101] have concluded from a recent study that the antitoxin for diphtheria which Schick showed was present in the blood and tissues of many human beings, was transmitted passively from mother to infant through the placenta. The facts so far collected, regarding the familial tendency of idiosyncrasy to foreign protein, do not accord absolutely with those observed in experimental transference of immunity and anaphylaxis from mother to offspring. In the first place, sensitization in man is

not transient but often of years' duration. In the second place, it may occur through four generations, and in the third place, it is often noticeable principally or solely, as occurred in the extraordinary family, described by Laroche, Richet and St. Girons,[102] in the male members. And finally, the sensitization may not always be to the same protein. In at least one family which we studied the father was sensitive to horse serum and the son to egg-white. If inheritance is a factor, therefore, it cannot be by means of passive transfer from mother to infant, but in some instances at least may be a true inheritance of cell characteristics derived either from the father or mother. The whole problem, since it is one of greatest importance, needs careful study, but one is almost inclined to suggest that occasionally sensitization towards foreign protein may be an inherited characteristic of the cell plasm and often not highly specific in character. Whether this is dependent upon the presence of true antibodies to the foreign protein or ferments, as Abderhalden and others have suggested, it is impossible to say. That receptors in the sense of Ehrlich, for sheep cells may reside in the organs of such species as lobsters, crabs, chickens and dogs, has been shown by Amako,[103] and more important still is the demonstration of von Dungern and Hirschfeld [104] that the iso-agglutinin are inherited and are transmitted strictly according to Mendelian law.

The high degree of susceptibility in some people with spontaneous sensitiveness, the multiplicity or lack of specificity of sensitization and the distinct tendency for it to occur in families differentiate these individuals from the artificially sensitized, and suggest that there is some unknown factor here which is absent in men and animals subjected to artificial sensitization.

The introduction of foreign protein, however, results in essentially the same reaction in both instances and individuals in this state of spontaneous sensitization suffering from the effects of contact with protein ordinarily harmless must clearly be differentiated from the normal person who becomes ill from the absorption of one of the poisonous products which may through various means be split off from the protein molecule.

Undoubtedly certain violent intoxications in man are caused

by the entrance into the body of these toxic products of protein digestion and it is possible that they may give rise to symptoms which resemble the true anaphylactic shock. The rupture into the peritoneum of an echinococcus cyst is frequently attended by violent symptoms of collapse which Weinberg and Ciuca [105] have ascribed to anaphylactic shock. But as it is difficult to sensitize animals to the fluid of echinococcus cysts, or to show that animals or men carrying echinococcus cysts are sensitized to the fluid, and since the fluid itself is toxic, Graetz [105a] concludes that the symptoms after rupture are not due to anaphylaxis but to the absorption from the peritoneum of toxic products split from the protein of the cyst.

The investigations of Whipple [106] and of Murphy and Brooks [107] upon the acute poisoning in dogs whose duodenum has been closed off in a loop, have shown that the symptoms are due to a toxin absorbed through the duodenal mucosa, and recently Whipple, Rodenbaugh and Kilgore [108] have been able to isolate this toxin and show, as they believe, that it is proteose. It may be therefore that the acute collapse and toxic symptoms that accompany some of the severe gastro-intestinal disturbances are caused by the absorption of proteoses or such substances as histamin. These poisonous substances may be taken into the gastro-intestinal tract with the food, especially if it is spoiled, or they may be present there already. Such attacks of food poisoning are perfectly familiar, but it is questionable whether they result in sensitization and they should not be confused with anaphylaxis.

So far our attention has been concentrated wholly upon the susceptibility of man to the proteins of animals and the higher plants or their split products, but it is necessary now to refer at least briefly to a somewhat different form of susceptibility but one of great importance, namely, "allergy," or changed reactivity, or susceptibility to infection.

The principle is beautifully illustrated by the classical experiments of von Pirquet on vaccination. Cutaneous inoculation of the normal individual by the virus of vaccinia results in the appearance after an interval of 9 to 12 days of the typical skin

lesion or local vaccinia, the normal reaction. Vaccination of a person recently immunized to cowpox shows within 24 hours the appearance of a small vesicle surrounded by a red areole which rapidly fades, and revaccination after several years results in a positive "take," which, however, appears early on the 6th or 7th day instead of the 9th or 12th day as it does in the normal individual. The similarity to the normal immediate and the accelerated reactions on inoculation and reinoculation of horse serum is very striking and was immediately apparent to von Pirquet.

The relationship which anaphylaxis bears to this broad field of immunity and susceptibility and the part which the poisonous products of protein digestion may have in causing the symptoms of infections, such as fever, was thoroughly discussed by Dr. Vaughan two years ago, and I shall therefore confine myself to one phase of the subject which will still repay study.

A method of studying the allergy to infection in man which has been developed within the last few years is the altered local reaction to the conjunctival, subcutaneous or intracutaneous injection of bacteria or their extracts. The reaction of the conjunctiva and the skin of tuberculous patients to tuberculin is perfectly familiar. Similar specific reactions have been obtained with the extracts of bodies of the infecting bacteria in such disease as glanders, typhoid fever, syphilis, the trichophytic infections and lobar pneumonia. In general it may be said that the response to the injection of these organisms or the extracts appears either during the course of the infection or after recovery of the individual. The tuberculin reaction according to most observers appears in animals fifteen to twenty days after infection by the tuberculin bacillus. The typhoidin reaction of Gay can be obtained many years after recovery from typhoid fever. Mueller, Gachtgens and Aoki,[109] who have studied the development of the mallein reaction in glanders in horses, state that the skin test may give a positive result on the 4th or 5th day after infection, and Weil,[110] who has recently described a cutaneous reaction obtained in a certain proportion of cases of pneumonia,

when the autolysates of pneumococci are employed, states that the reaction first appears after the crisis.

The pathology of all these reactions is very similar, and though the histology of the lesion has only been studied extensively in tuberculosis, the few observations which have been made of the other reactions indicate that they resemble one another very closely and consist principally in the infiltration of the subcutaneous tissues by mononuclear cells which are collected about blood-vessels. Unlike the reactions obtained with animal and vegetable proteins in sensitized individuals, these reactions do not come on within the first few hours after inoculation, but make their appearance first in 20 to 48 hours. The tuberculin reaction may be delayed for 2 to 3 days and the luetin reaction for 10 to 21 days.

It is possible that similar local forms of allergy occur spontaneously during the course of many diseases. In the cases of chronic infections by the Streptococcus viridans the cutaneous hemorrhages and painful nodules have been supposed to result from emboli to the skin, but Dr. Lamb in studying a few of these lesions in serial section has been unable to find thrombosed vessels. The histological picture is again not unlike that seen in the tuberculin reaction. Quite similar is the change in the glomeruli of the kidney so common in these cases and so unlike the acute suppurative processes usually encountered in acute infections, that it is possible it may represent an allergic reaction towards the infecting organism.

Faber [111] has recently shown that a previous injection of streptococci into the joint of a rabbit will alter the local resistance of the joint in such a way that intravenous injections of that type of streptococci which in normal animals rarely, if ever, affect the joints, invariably produce in the treated animals a localized infection of the joints in these animals. This local allergy seems, too, to be specific. Finally, one may cite the transition of an acute local infection to a chronic local inflammation as a beautiful example of allergy or changed reactivity to an infecting organism. It is worthy of notice, too, that many of these local allergic processes are associated in human beings with the appear-

ance of mononuclear cells. In rabbits the polymorphonuclear leucocytes predominates both in the tuberculin reaction and in that obtained with animal proteins.

During the artificial immunization of both man and animal by repeated subcutaneous injections of bacteria or their extracts, both local and general reactions occur which must be interpreted as an evidence of allergy. It is generally recognized that severe local and general reactions are much more likely to occur in patients who have had typhoid fever or who have been subjected to previous inoculation than in normal individuals, and that the local or general reaction in normal persons is more likely to be severe following the second or third inoculation than after the first. Nichols [112] states that severe local reaction occurs in 50 per cent. of cases after repeated inoculation. In very rare instances the general reactions following large injections of bacterial vaccines spaced at long intervals are immediately followed by alarming collapse, swelling of the face, dyspnœa and suppression of urine, a condition not unlike anaphylactic shock.

Indeed, many of these conditions have been ascribed to sensiization, likened to anaphylaxis and accepted as such. Bacterial bodies contain nucleo-proteins, the proportion of nitrogen varying in different bacteria from 5 to 10 per cent., and it cannot be doubted that it is possible to produce anaphylaxis with bacteria or their products, though the primary toxicity of these suspensions and extracts is often so great that the results of many experimenters leaves one in doubt as to whether the effect of injection is really that of anaphylactic shock or some other form of rapid intoxication. In working with bacteria the results are always complicated by the presence of poisonous substances, whatever their origin, and until it is possible to obtain from bacterial bodies proteins free from the toxic split products, or substances developed from bacterial growth, it will be difficult to determine what part the true protein sensitization and the anaphylactic reaction plays in susceptibility and infection.

Even the extensive studies upon tuberculosis have not completely explained the reactions which Koch's O. T. tuberculin, which is a glycerin broth filtrate, calls forth. In general, the

reactions produced in the tuberculous individual differ very slightly from those obtained with bacillus emulsion or extracts rich in protein, but with the latter animals may be readily sensitized, while with the former, though such sensitization has been accomplished, it is with great difficulty, and the reactions according to Lewis [113] are dependent upon the protein in the broth rather than in the extract from the bacilli. Though Baldwin [114] showed that animals may be sensitized with tuberculo-protein as with other bacterial proteins, most workers have failed to sensitize guinea-pigs passively with the serum of tuberculous patients or animals. Austrian [115] has shown that such a thing is possible if tuberculo-protein instead of Koch's O. T. tuberculin is used as an antigen. Animals actively sensitized to tuberculo-protein may give a very faint skin reaction to O. T. tuberculin, though the infected animals, of course, react strongly. In fine, the skin reaction and the general reaction to O. T. tuberculin seem to depend upon the presence in the body of an active tuberculous focus, and Bail,[116] in transplanting tuberculous tissue from a tuberculous to a normal animal, found that the reaction after the injection of tuberculin always takes place in the tuberculous tissue. It is evident, therefore, that though the reaction in the tuberculous patient or animal is the same whether tuberculo-protein or O. T. tuberculin is employed, the anaphylactic reactions differ greatly and cannot be obtained with the latter.

To what extent the forms of allergy such as the diagnostic skin reaction in infectious processes are dependent upon protein sensitization, and can thus be accredited to true anaphylaxis, is uncertain, but before sweeping conclusions can be made upon the identity of allergy and anaphylaxis much careful experimental work is necessary.

The analogy is very close, however, and since one of the striking features in infectious processes is the response on the part of the body by exudation and proliferation of cells, namely an inflammation, it is important to determine whether similar processes accompany or follow anaphylactic reaction to simple proteins. The characteristic result of acute shock in guinea-pigs is the appearance of hemorrhages. But the guinea-pig that has

recovered from a single shock shows no permanent trace of these lesions. Repeated injections of serum made subcutaneously in rabbits, on the other hand, give rise to œdema, hemorrhage and necrosis, as was pointed out by Arthus, and the lesion is characterized according to Schlecht and Schwenke [117] by an exudation of mononuclear cells and eosinophilic leucocytes. Friedberger [118] and Ishioka [119] showed, too, that horse serum sprayed into the trachea of sensitized guinea-pigs produced a cellular type of pneumonia, the exudate into the alveoli being rich in mononuclear cells and eosinophiles. We [120] have found that repeated intravenous injections of horse serum and egg-white in sensitized guinea-pigs, rabbits, dogs and cats, brought about focal necroses in the liver, kidney and heart muscle which was followed by an exudation of mononuclear cells. In over 80 per cent. of the animals changes were found in the kidneys which were often so extensive that they led to an advanced form of nephritis.

Though it would not be justifiable to apply the results of such experiments to disease in man, it is interesting to note that one of our patients who showed spontaneous sensitiveness to several proteins from plants and especially to phaseolin, and who had had several violent attacks of asthma, urticaria and diarrhœa after eating beans, developed a pronounced and persistent albuminuria and cylindruria following such an attack one year ago. Undoubtedly infection is the most important factor in the cause of nephritis in man, but such observations suggest that the progressive process in the kidney may depend upon an allergy or attend susceptibility of this tissue towards the bacterial protein.

It is known that about 10 per cent. of patients with serum sickness have a mild degree of albuminuria and show both hyaline and granular casts in the urine, and it was, therefore, with much interest that Dr. Rackemann and I studied the functional activity of the kidney during the course of this disease. Most of the patients have developed their serum sickness after the use of large amounts of antipneumococcus serum, which Dr. Cole has kindly furnished us for the treatment of pneumonia caused by the pneumococcus type I. In this study attention has been paid especially to the excretion of phenolsulphonaphthalein, water and

sodium chloride. The nitrogen metabolism is so much disturbed during and after an attack of pneumonia that little significance can be attached to the changes in the total non-protein nitrogen of the blood. The coëfficient of urea excretion, however, employed by Ambard, has been studied by the recent modification in technic described by McLean,[121] and we have not found that this is greatly modified during serum sickness. The excretion of chro-rides and water, on the other hand, is often profoundly affected. The changes that take place in the elimination of salt and water during convalescence from an attack of pneumonia, that has not had serum, are as follows: During the attack the excretion of water is low and the chlorides are eliminated in small amounts and low concentration. Shortly after the attack the retained chlorides are excreted in excess of the intake and in high concentration, but on a fixed water and salt intake soon come to a normal balance. We have not found that this course is materially interfered with by such febrile complications as empyema. In the serum sickness that may follow the use of antipneumococcus serum and in one case after the intraspinous injection of anti-pneumococcus serum, the ordinary course of the elimination of water and sodium chloride was greatly disturbed. With the onset of serum disease the excretion of water diminished rapidly and with it the elimination of sodium chloride. One important point, too, is that the patient is unable to concentrate the sodium chloride, which may fall below 0.2 per cent., a very low figure. The excretion of phthalein is in a few cases slightly affected as well and with the appearance of albumin and casts in the urine and the development of œdema, the patient presents a clinical picture very closely resembling a mild case of nephritis of the salt retention type. Sufficient studies have not been made to determine whether this salt retention is in the blood or tissues, but at all events, it is usually accompanied by œdema. These alterations, so far as we have observed them, are purely transitory, and with the subsidence of the attack the patient and the renal function return to a normal state.

Such studies are designed primarily to throw some light upon the possible importance of repeated anaphylactic shocks in the

spontaneously and highly sensitized individual, and it would be the greatest mistake and misfortune at the present time if they were allowed to have any bearing on the use of antitoxic and antibacterial sera. The concentrated diphtheria antitoxin, such as is employed now, rarely produces serum disease or sensitizes sufficiently highly to make a second dose, particularly if it is given subcutaneously, in the least dangerous, and it is only to the spontaneously sensitive who react to the first injection that harm is likely to come.

To prevent accidents in such unexpected instances, especially if large quantities of serum are given intravenously, a preliminary intracutaneous injection of 0.1 to 0.01 c.c. of serum should be made to determine whether or not the patient is spontaneously sensitive to the serum which is to be employed.

Though it would be interesting to recount the methods which have been employed to desensitize both artificially and spontaneously sensitized individuals, this important problem in therapeutics must be left, since there is not time to do it justice, and in conclusion I shall point out the conditions which such methods must combat.

The injection of foreign proteins in man brings about the same condition of hypersensitiveness towards subsequent injections that it does in animals.

Certain individuals may show spontaneous hypersusceptibility to one of several foreign proteins. These people differ from the artificially sensitized in that their susceptibility is very great, is shown towards several different proteins and has a tendency to occur in families. And finally, that this state is associated with and directly responsible for some well-defined pathological conditions.

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SOME PHASES OF THE NEPHRITIS PROBLEM*

PROF. HENRY A. CHRISTIAN, M.D.

Harvard University

SOME of my predecessors as lecturers before the Harvey Society have selected topics of which it was impossible to present a quite comprehensive review of contemporary knowledge within the limits of such a lecture; while others have had personal investigations to report of such completeness and importance that their presentation fulfilled amply the requirements of the occasion. My subject and my own work fall within neither of these groups. From the vast literature on nephritis no reviewer could condense into the space of an hour any adequate presentation of the results of the many valuable investigations of the past, while my own studies have not a value deserving the occupancy of an hour of your time.

I have chosen to speak of only certain phases of the nephritis problem illustrative of the present-day viewpoint of the subject and indicating the trend of recent investigation. In doing this I will speak relatively more of the work during the past eight years of myself and my associates in the laboratory of the Department of Medicine at Harvard and the Medical Clinic of the Peter Bent Brigham Hospital than of the work of others because I am more familiar with our own work, not because I have the opinion that it is of proportionately as great value as many of the splendid researches that have come in the last decade from other laboratories and clinics.

From the time of Bright's publication in 1827 with which began any real knowledge of nephritis down to the late nineties almost all investigations concerned themselves with renal structure in nephritis or with statistical study of symptomatology. In these studies structure rather than function was predominately the point of interest. A conception of the relations of the diseased kidney to the patient's symptoms was evolved in large part from an examination of kidneys from men dead of nephritis.

Hypotheses as to the probable relations of these pathological changes to observed symptoms and to disturbances in urinary excretion were formed.

Beginning in 1900 interest shifted toward animal experimentation. Renal function in nephritis, whether produced experimentally in animals or arising naturally in man, replaced renal structure as the problem of greatest interest. A considerable proportion, however, of this interest in renal function was founded on the hope of a more satisfactory correlation of renal function as observed during life with changes in renal structure as found after death; a hope as yet unrealized, in fact, almost given up as an unattainable goal.

In 1907 I became interested in the problem of nephritis and since then have devoted such time as was available from medical teaching and the conduct of a hospital service to the study of some of these problems. In this study I have associated with me a number of men, R. M. Smith, Walker, O'Hare, Dawson, Fitz, Frothingham, Peabody, Smillie and Woods, whose independent work I will describe in connection with my own.

We began with a few of the problems of experimental nephritis. Our first study, the work of R. M. Smith,[1] was on the origin of urinary casts. Two views prevailed; one that all casts originated from disintegrated tubular epithelium, the other that some of them at least were formed from albumen which escaped through the damaged glomeruli. Acute types of experimental nephritis furnished excellent material for the study of this problem, inasmuch as the urine might be examined in relation to renal lesions of varying severity and age and at any period the kidney might be studied histologically. From our work it seemed very probable that all casts were composed of material primarily coming from the tubular epithelium and that granular casts were relatively young casts, hyaline casts older in the sense of requiring a longer time for their preparation. It did not seem very probable that albumen excreted from the glomerulus in solution would coagulate or precipitate in the tubule, though this mechanism might easily take place with fibrin casts if such really ever occurred in the urine.

The work of Schlayer and his co-workers at about this time had aroused much interest in the functional disturbances resulting from experimental acute renal lesions. They had differentiated the two main groups of disturbances, the tubular and the vascular, and had outlined the functional disturbances characteristic of each. In their work they had emphasized the frequent discrepancies between functional change and demonstrable anatomical lesion, especially in the group of vascular nephritides. O'Hare and myself undertook a careful study of the finer histology of some of these experimental lesions and were able to show that in the vascular group glomerular lesions were more common than Schlayer and his co-workers had found and that they occurred in a considerable variety of forms. One of these lesions consisting of the deposition of fine hyaline granules in the wall of the glomerular tuft was a type of glomerular degeneration previously undescribed,[2] while others of them were similar to lesions sometimes or often found in human kidneys.[3] Still there remained a definite discrepancy between functional disturbance and anatomical change in the kidney, and our histological studies did not throw much light on the nephritis problem in man, whether looked at from a functional or from a structural viewpoint.

Experimental acute lesions lend themselves well to study with functional tests. It was interesting to see the relation of the phenolsulphonephthalein test to the non-protein nitrogen or urea nitrogen of the blood; the phtalein output quickly drops, the blood nitrogen more slowly increases; the former expresses the immediate functional condition of the kidney; the latter measures the result of the past and present hindrance to renal excretion.[4] In the same way the amylase in the urine was studied by Fitz[5] in contrast to the urea nitrogen of the blood. Fitz found that amylase followed much the same curve of excretion as did 'phthalein but as a test for renal function was less delicate than the 'phthalein test. As had been done by Schlayer, water, salt, lactose and potassium iodide excretion were studied. Using experimental lesions, since their severity can be controlled very accurately, a good understanding of several of these functional tests and their relative values was obtained by us and by others

more quickly than would have been possible from the study of human acute nephritis where much time would have been lost in waiting for the necessary number of suitable cases to turn up, and so for experimental study we were in a better position to apply these tests to human cases.

Equally well are the acute experimental lesions adapted to the study of the effect of diuretic drugs. O'Hare, Walker, Dawson and myself have investigated in this way theobromine sodium salicylate,[6] theocin, caffein, potassium acetate and water,[7] and found that all tended to shorten rather than prolong the life of animals with severe acute uranium nitrate nephritis. Using 'phthalein as a measure of renal function we were unable to discover any definite constant improvement in renal excretion following the use of theobromine sodium salicylate. Over short periods of time with animals anæsthetized with urethane Fitz[8] found that with an acute renal lesion produced with uranium nitrate, theobromine sodium salicylate and theocin caused an increased output of water, sodium chloride and nitrogen and did not diminish 'phthalein excretion; while with a lesion produced with potassium bichromate there was no increased output of nitrogen and 'phthalein excretion was diminished when there was a mild renal lesion. When the lesion was severe the 'phthalein usually indicated that the diuretic had made the function of the kidney worse.[9] These diuretic substances quickly lead to renal fatigue with decreased excretion. Our experiments as a whole indicate harm rather than benefit from giving diuretic drugs in acute nephritis.

Very interesting observations were made on the relation of potassium excretion to renal lesions. In one of our patients with chronic nephritis it was noticed that a substitution of potassium chloride for sodium chloride in a dietary test of renal function produced toxic symptoms. In animals with acute experimental renal lesions Smillie[10] found that a stage was soon reached, measured by a non-proteid nitrogen value in the blood of 100 mg. per 100 c.c. of blood, in which the kidney was very slightly permeable to potassium salts. Under these conditions potassium salts were markedly toxic, rapidly causing the death of the animals, though an animal with a normal function could tolerate large

doses of potassium without any evidence of injury. The rapid excretion of potassium with an intact kidney prevented any evidence of toxicity; delay excretion by a renal lesion and toxicity at once became evident. These studies indicate that large doses of potassium iodide, for example, might be definitely injurious in a patient with markedly impaired renal function.

In man chronic rather than acute renal lesions are encountered most often, and the problems of chronic nephritis receive the greatest attention from pathologists and clinicians. Could one produce with regularity in animals various types of chronic renal lesions analogous to those found in man, many problems could be studied by methods not applicable to the human being. To produce such a chronic nephritis experimentally many efforts have been made. Like other experimenters we have been able to produce in animals, chiefly rabbits, lesions of a chronic nature with connective tissue overgrowth very similar to those found in the types of chronic nephritis seen in man. With repeated sublethal doses of uranium nitrate and of potassium bichromate, chronic experimental lesions were produced by Smith,[11] and similar, possibly more constant, results were obtained by O'Hare[12] with a combination of a chemical substance (uranium nitrate) and bacteria (B. coli communis). These lesions were quite similar to those obtained by other observers using a variety of substances as causative agents, but all alike fail of the intent held by most workers; namely, to produce regularly chronic lesions capable of functional study with a view to throwing some light on the cause, the symptomatology and the treatment of chronic nephritis in man. The kidney of animals possesses a surprising power of regenerative repair which makes uncertain the production of chronic lesions. Moreover, however close the resemblance, it cannot be said that in animals a condition identical with chronic nephritis in man has been produced as yet by any experimenter. Finally the methods usually employed for the production in animals of such chronic lesions are at best only remotely related to any possible causative factors of chronic nephritis in man.

To help in any understanding of human chronic nephritis, chronic lesions must be produced in animals with great regularity

and they must cause in the animal changes of a nature similar to those observed in man, such as vascular hypertension, cardiac hypertrophy, dyspnœa, œdema, uræmia, etc., before such information can be gained about chronic nephritis from animal experimentation. Looked at in this way all work on experimental chronic nephritis falls short, though some of the phenomena mentioned above can be produced. However, it does not seem impossible that in some animal a true chronic renal lesion with the typical secondary conditions that go to make up the picture of chronic nephritis in man should be produced by experimental methods of investigation. Such an accomplishment would facilitate undoubtedly the unraveling of the nephritis problem in man, and its value would be so great that it is well worth while to continue to strive for its attainment.

Though the experimental studies of Schlayer and his co-workers [13] differentiated two very distinct types of renal lesion, the vascular and the tubular, and though with their methods of study each type was shown to cause very characteristic disturbances of renal function in animals, the value of these studies has been rather in re-arousing interest in the investigation of renal function in human nephritis than in furnishing us with the satisfactory functional classification of renal lesions in man. In each case of human nephritis both tubules and vascular apparatus usually are involved. The one structure or the other may be disturbed in greater amount, but it has not been possible, except in very few cases, to separate the patients with any sharpness into cases with tubular lesions and cases with vascular lesions in the sense of Schlayer and his co-workers. Two of the substances which they used for functional testing in animals have hardly in the human being measured up to the usefulness which might have been anticipated from the results in animals or which was claimed at first from their use in man. Schlayer utilized the time required for the excretion of a given amount of potassium iodide as an index of tubular efficiency and the time and amount of lactose excretion to indicate the efficiency of the glomeruli. We, like other observers, have used these substances in a very

considerable number of patients, but gradually have discarded them as furnishing comparatively little useful information.

Widal and his co-workers [14] studied the renal excretion of sodium chloride and nitrogen and grouped their cases of chronic nephritis into those with deficient power to excrete sodium chloride, which cases usually showed œdema as a chief symptom, and those unable to excrete nitrogen readily in which uræmic manifestations generally were prominent. Our study of salt and nitrogen excretion except in an occasional case has not yielded any sharp differentiation of patients. There is an occasional patient with a marked inability to excrete sodium chloride who becomes œdematous when sodium chloride intake exceeds sodium chloride output and whose kidney shows but little if any impairment of its function to rid the body of nitrogenous substances, but such cases often are not, strictly speaking, cases of nephritis, but rather patients with disturbed salt elimination and no more to be regarded as nephritis than would be cases of diabetes mellitus or diabetes insipidus. If the typical cases of chronic nephritis, whether œdema is a prominent feature or not, are investigated with respect to salt and nitrogen elimination, it has been our experience that in most patients both show delayed excretion; in the earlier stages salt excretion rather than nitrogen excretion is disturbed, but as the disease progresses nitrogen excretion becomes increasingly delayed until in advanced cases both salt and nitrogen excretion are markedly and about equally disturbed.[15] We have not encountered cases of the type described by Widal with normal salt excretion and delayed nitrogen excretion.

In all of the earlier functional studies of patients with nephritis the desire to make an anatomical diagnosis has been prominent; sometimes it has been the acknowledged goal, at other times, though not so stated, it is evidently the aim of the investigator. Little by little it has become recognized that such an attainment has not been approached with any closeness and I think by most investigators it is now regarded as improbable that we will ever be able to correlate closely postmortem anatomical appearances with the functional disturbances of the kidney

during life, at least so long as present pathological technic and classification continue in use. Improved methods of studying renal lesions, of course, may change these conditions at any time. In a number of patients we have had the opportunity to carry out a group of functional renal studies and on the death of the patient have submitted the kidneys to pathological examination.[16] In these patients there was no evident relation to be made out between anatomical changes in the kidney and antecedent functional disturbances in any selective sense that would justify an anatomical classification. In our experience in a functional sense patients with nephritis do not separate themselves into distinctive groups; rather is it indicated that there is a progressive increase in functional disturbance with advance of the lesion, though there is an undoubted tendency for certain cases to show continuously a much more marked impairment of function as measured by one set of tests than by another, indicating that functional disturbances depend on selective excretory activity and that were not the various renal structures pretty generally involved in nephritis a more definite classification based on tests of renal function could be made.

However, at the present time tests of renal function are of more value for indicating the presence of renal lesion, for measuring its extent and for indicating its management than as a means of classification of cases. For these purposes they add greatly to the value of our clinical study of patients with nephritis. Out of the very numerous methods of testing renal function certain ones have survived either by reason of ease of application or by reason of yield of information in proportion to the amount of labor they require. Some have been discarded because the same information may be obtained from simpler procedures, or from ones requiring less complicated and expensive apparatus or occupying less time in their carrying out. Others have been given up because they caused more discomfort and inconvenience to the patient than some other one yielding the same information. Those that are still in use, though they yield much valuable information, are not thoroughly satisfactory and better ones probably

can be worked out with our increasing knowledge of renal function under varying conditions in man and animals.

The functional tests now most generally used are the ones that show the power of the kidney to excrete a dyestuff and those that measure the efficiency of the kidney in the excretion of water, salt and nitrogen. Of the former group the phenolsulphonephthalein test of Geraghty and Rowntree [17] very largely has superseded all others as being the best of these tests owing to its simplicity and its accuracy.

To determine the excretion of water, salt and nitrogen, several methods are employed. These substances are quantitated in the urine in relation to a fixed dietary intake; or they are quantitated in the blood; or the relation between the amount in the blood and the urine is expressed in the form of a formula of excretion.

The impetus more recently to the study of urinary water, salt and nitrogen in relation to dietary intake we owe to the German clinics, especially to von Monakow [18] and to Schlayer and Hedinger.[19] Two general plans have been followed. By one of these the patient is on a fixed diet containing a known amount of fluid, salt and nitrogen. By quantitating these substances in each twenty-four-hour amount of urine the promptness and completeness of excretion of the added salt and nitrogen are determined. Delay in excretion or incomplete excretion is indicative of disturbed renal function.

By another plan on one day the patient has a standard mixed diet containing definite amounts of salt, nitrogen, water and purin bases, in certain of the meals little, in others a considerable amount. The urine is collected in two-hour portions and the salt, nitrogen and specific gravity of each portion is determined. The curves of excretion as plotted from the values so obtained in comparison with the excretion from normal kidneys indicate departure from normal renal function.

The determination of these substances, salt and nitrogenous bodies, in the blood has been made by numerous observers in the past but the simplified methods of Folin [20] and of van Slyke [21] recently have stimulated and made possible many new and excellent investigations of this phase of the subject.

To Ambard [22] is due most credit for our present interest in the rate of excretion of nitrogen and salt as determined from formulæ which take into account the concentration of these substances in blood and urine, rate of urine flow and weight of the patient. Ambard and his co-workers have worked out many of the laws governing these excretions and have shown their constancy under normal conditions. McLean,[23] by a slight variation in the structure of the formulæ of Ambard, has expressed the excretion as a numerical index which has some advantage over Ambard's way of expressing his results. Van Slyke's urease method of determining urea has rendered more accurate the determinations and this method has been used by McLean to give a greater constancy to his figures than would have obtained with the older methods of determining the urea. However, it is only fair to Ambard and his co-workers to say that the work of others has amplified rather than corrected the conclusions drawn by Ambard from the studies made by himself and his associates.

The work of all of these observers is open to the criticism that each observer has used, as a rule, but a single method of studying renal function, usually the method devised by himself, and he has not compared often the results that might be obtained by applying to the same patients several methods of study. Furthermore, it is surprising how few of the cases studied by functional methods have had checking up from postmortem anatomical study. We have already called attention to the unsatisfactoriness of the correlation between histological changes in the kidney and prior functional renal study. Still the autopsy checking up of results is very salutary and of course excludes certain errors such as the diagnosing of a simple chronic passive congestion as a chronic nephritis. It is to be remembered, however, that most of the fatal cases will have had functional study under the handicap of the seriousness of the patient's condition hindering the carrying out of many functional studies and really represent the function of the late stages of a nephritis in relation to postmortem findings. As already stated, we have had a certain number of cases for functional study and later have had opportunity to examine their kidneys. However, in almost all of these the dietary

tests were impossible of carrying out satisfactorily owing to the advanced stage of the disease, and so the studies of renal function were incomplete. It will be necessary to make studies of renal function at intervals during the course of nephritis and then on the death of the patient study the kidney histologically before a satisfactory verdict can be rendered on the relation between renal function and renal structure as shown by the pathological study of the terminal stage of the nephritis.

We have been able to study a considerable number of non-fatal cases with a variety of methods of testing renal function, such as the phenolsulphonephthalein test, the added urea and salt dietary test, the test renal day, the determination of the indices of urea and salt excretion, etc.[24] Some of these tests can be carried out almost simultaneously; others of them require several days and only one at a time can be done. For these reasons there is the possibility for variations in the patient's condition influencing renal function so that in comparing the results of different tests conditions are not always identical. Still in most cases there is a surprisingly close parallelism between the results obtained from different methods of testing renal function. Almost always a low 'phthalein output is accompanied by an increase in urea or total non-protein nitrogen in the blood and the index of nitrogen excretion is proportionately lowered below the average normal figure. In these cases dietary tests of any sort show delayed excretion of nitrogen and salt with fixation of specific gravity and percentage concentration of nitrogen and salt. Occasionally there are exceptions and one set of tests indicates less disturbance of renal function than another set. In some of these patients these variations are obviously due to extra-renal causes or changes in the patient's condition during the course of the testing. There remain, however, a few cases where no explanation can be found for failure of the results of different tests to agree and for this reason alone, it is important not to confine one's study of renal function to too few tests, however satisfactory the individual test would seem to be.

Patients who show these marked disturbances of renal function as measured by several tests usually are quite evidently seriously

ill patients when studied merely by the regular routine methods of history of symptoms and simple physical examination. They are undoubted cases of advanced chronic nephritis and there is no question as to diagnosis; renal tests are not needed to make the diagnosis and really do not help from that viewpoint. Nevertheless, by using tests of renal function some cases are found which, while showing marked disturbance of renal function, have but few if any symptoms and appear to be but slightly sick individuals. Their poor renal function comes as a great surprise to the clinician in charge. Here the tests are of much use in making a prognosis and enable the physician to warn his patient of impending catastrophe. Although he may be unable in any way to prevent this catastrophe, foreknowledge of it may be of incalculable value to the patient and family. In our experience in such cases the progress of the disease has borne out the accuracy of the information from the tests of renal function provided they have not been made during a period of acute exacerbation of renal disturbance or when renal insufficiency is aggravated by an increased, though temporary, circulatory insufficiency. In cases in which there is any suspicion of these conditions the renal tests should be repeated after an interval. In our experience, however, acute exacerbations of renal disturbance and circulatory deficiencies are evident in studying almost all of the cases and should not form any appreciable source of error in interpretation. Obviously tests of renal function like all clinical tests should not be exalted into a fetich; without an admixture of brains and common sense they can lead to absurd conclusions with the man who is willing to stake all on a single test. A recent excessive ingestion of proteid by a patient with a stricture of his urethra but otherwise healthy could easily simulate a marked renal insufficiency by several tests and mislead that type of clinician who, with but a scant glance at his patient and no conception of the patient's symptoms, rushes to his laboratory tests with the idea that scientific accuracy increases by the square of the distance from the bedside and by the cube of the time spent in carrying out a test out of touch with the sick man. This would seem so

evident a proposition as not to need repetition, and yet I regret to say that I have seen almost this identical mistake made.

As I have pointed out earlier in this paper, tests of renal function have a value for three purposes: for diagnosing the presence of renal lesion, for measuring its extent and for indicating its management. For diagnosing the presence of marked renal disturbances the tests as just pointed out are not of great value because the existence of marked nephritis is usually evident without them. In the earlier stages of nephritis tests of renal function are of considerable value in diagnosis. It must be admitted that albumen and casts in the urine are among the most delicate indicators of disturbed renal function. When found the question arises, do they indicate a disturbed function of a type to be regarded as a nephritis that will progress? In these earlier cases phenolsulphonephthalein excretion is so nearly normal as to be of no diagnostic aid. Non-protein nitrogenous bodies in the blood are well within normal limits and their determination does not help. It is in these earlier cases that the dietary tests indicate distinct disturbance of the type found in definite chronic nephritis, though the disturbance is less evident than in a somewhat more advanced stage of the lesion. Delay in excretion of sodium chloride, a tendency to fixation of specific gravity, hypersensitivity with polyuria or rapid fatigue, all are suggestive of an actual nephritis in the patient with slight albuminuria or cylindruria. We have found just these changes in cases in which phenolsulphonephthalein output was good and blood urea was normal, but in which symptomatology, slight œdema, moderate hypertension, etc., seemed to clearly indicate that they were cases of early chronic nephritis. Very likely without this collateral evidence a diagnosis of early chronic nephritis should be made on patients with slight albuminuria and these changes just enumerated as indicated by dietary tests. These cases very frequently show, too, a lowered index of urea excretion and often a plus salt balance, and these methods are of equal value with the dietary tests.

We should not be too dogmatic, however, with regard to the diagnosis of early stages of chronic nephritis by renal dietary

tests or determinations of the indices of urea and salt excretion. Almost all of these studies have been made very recently and one is not justified in saying that a patient with these slight disturbances of renal function is in an early stage of nephritis until many such cases are tested and watched and retested over a period of five or ten years and found to develop with great regularity into typical cases of chronic nephritis, which means that they show themselves to have a progressing renal lesion ultimately fatal and showing at the postmortem examination an anatomically demonstrated chronic nephritis. We are in danger in present enthusiasm to overlook the fact that in the study of a chronic disease almost no method can have a proved value until much time has elapsed. Such a proved value certainly cannot be attached at present to these tests of renal function.

What are the relative values of dietary tests such as the added salt and urea test and the dietary renal day as compared with the determination of the indices of urea and salt excretion? I do not believe that we are in a position to give an answer now. As I have already pointed out, in our patients all the tests agree surprisingly well, but we have not observed long enough the very early cases to be justified in holding any definite opinion as to relative value. The dietary tests are time consuming for both patient and laboratory worker, unless they can be simplified materially. Their inapplicability to ill patients unable to take the diets does not apply to early cases but other objections do. The determination of the indices of excretion is quicker for the patient at least and simpler in not requiring hospital observation and a weighed diet. It seems to me, however, that variations in the indices in relation to variations in water output are not sufficiently well understood to justify our feeling completely satisfied with them. Then the occasional abnormally high indices that we get have not received satisfactory interpretation; they, too, require the time element and repetitions from period to period to be understood. So I feel that both methods of testing renal functions should be persisted in for a much longer time before we are justified in giving up either one in favor of the other.

What value have these tests in prognosis? It is in this con-

nection that we get most help from tests of renal function. The tests aid very greatly in determining the severity of renal involvement and so aid in making a prognosis. The prognosis based on the tests, however, is one of degree of renal lesion rather than of duration of life. Duration of life depends on rate of progress of the lesion and the ability of the body to adapt itself to deficiencies in renal excretion, both difficult of foretelling. So until the tests indicate a very marked degree of renal insufficiency we can only surmise in a very loose way as to probable length of life. Repetitions of the tests at intervals give us some measure of rate of progress, but the course of development of the renal lesion in many cases undoubtedly is one of periods of exacerbation followed by periods of quiescence and little change. This decreases the accuracy of prognostic deductions from tests made at intervals. Still, tests of renal function do aid us materially in forming an opinion as to the probable period of activity of life of our patient and without them this becomes to a larger extent a matter of mere guess work.

For some time we have been planning the therapeutic management of patients with nephritis on the basis of the evidence from our tests of renal insufficiency. There seems little doubt but that a more appropriate degree of limitation of activity can be made with a knowledge of the condition of renal function. Dietary regulations based on the ability of the kidney to excrete water, nitrogen and salt as compared with the normal would seem to have a rational basis. If it is evident that the kidney excretes any of these substances slowly, or if fatigue appears shortly after excretion begins, the deduction made is that an excess of any of these substances in the food intake will overwork the kidney and probably increase the renal damage; on the other hand, a decrease in the intake will allow of a rest of renal function with either a gradual increase in power to excrete or a less rapid progression in the renal lesion. This is the hypothesis on which we work in planning a diet based on the ability of the kidney to handle water, salt and nitrogen. Long experience has indicated that an empirical reduction in intake of salt and nitrogen does benefit the patient. There has been much discussion as to whether

water should be increased or decreased. Limitation determined by renal function seems a more rational plan. Further trial and continued observation of patients alone can show how far this is true.

If salt is excreted poorly and there is œdema, reduction in salt intake usually decreases the œdema if it does not arise from circulatory deficiency, and this benefits the patient. When there is poor salt excretion but no tendency to œdema, does a salt excess in the food really act as a renal irritant in any sense? Probably it does, but I do not think our work so far has answered that question definitely.

In the same way, if nitrogen elimination is poor, a diet low in proteid leads to a decrease in blood urea and in total blood non-proteid and the index of urea excretion appears to improve, but we do not know how much this change really benefits the patient. We do not believe that these nitrogenous substances which we quantitate, urea, uric acid, amino-acids, etc., are injurious in themselves. We assume that when they are poorly excreted the actual toxic substances are retained in the body because in the uræmic or toxic state when presumably they are being retained we usually obtain high values for non-proteid nitrogen in the blood and have a low index of excretion indicating defective excretion of nitrogenous substances. We assume that the toxic substance is a nitrogenous body and so at the same time is poorly excreted. Very likely this is true, but as yet it is only an assumption and should be recognized as such. Decreasing food nitrogen, like decreasing food salt, very probably leads to renal rest and improvement in renal function and so to better excretion of toxic substances. Much careful observation, however, is needed as to the effect of dietary limitations on renal function before we can diet rationally our patients with nephritis.

Just the same thing holds with regard to the desirable amount of liquids for a patient with nephritis. In the patient with œdema, water reduction is indicated. Without œdema we are far from sure how to proceed in the question of the amount of fluid to be given. Observation of the relation between the amount of fluid intake and urine output helps us,

but in some cases definite renal hypersensitivity is present and we do not know the relation that exists between fluid intake and hypersensitivity with regard to renal fatigue, renal irritation and progress in renal damage. Here again much more careful observation is needed.

Our attitude towards dietary restrictions must be interwoven closely with that towards diuretic drugs. Water, salt and some of the nitrogenous constituents of food are diuretic in their action inasmuch as they increase urinary excretion. In considering dietary regulations we have emphasized the importance of considering the element of renal fatigue and of renal irritation in determining the advisability of certain dietary limitations. These same factors must be considered in regard to diuretic drugs. The effect of digitalis in improving cardiac function is one of our most brilliant therapeutic effects, and yet we know that with too long-continued doses of digitalis damage to cardiac function quickly results. For digitalis we have quite good indications as to when it is doing damage. Do diuretic drugs have any analogous relations to renal function? May they do damage to renal function? Have we indications of an injurious effect from them?

I have already pointed out that in acute experimental renal lesions we do have considerable evidence that diuretic drugs are harmful and there is very little evidence of a beneficial action. For chronic lesions animal experimentation gives us no satisfactory material for testing and so we are compelled to base our opinions on observations of patients with chronic nephritis in whom complicating factors render judgment more likely to be fallacious. Diuretic drugs under certain conditions can increase the output of urine. If there is present a considerable degree of œdema theocin and theobromine sodium salicylate will increase the amount of urine in certain patients. In our experience [25] this has been mainly in patients with cardiac insufficiency rather than renal insufficiency. Without cardiac insufficiency and with renal insufficiency diuresis in our experience usually does not occur from diuretic drugs even when the œdema is marked. This suggests that if the renal damage is in itself sufficient to cause œdema, diuretics are unable to stimulate the kidney directly or

indirectly to sufficient additional activity to increase the flow of urine. When a diuresis occurs what constituents are increased? Of course the output of water is increased. Some recent observations of ours show that with the increase in water there is a considerable increase in sodium chloride ouput, but relatively much less of an increased output of nitrogen. The removal of the excess of fluids from the patient's tissues is certainly beneficial, but their removal may not improve renal function. In fact, our observations show that following an active diuresis there may be for a day or two a decrease in renal function as measured by the index of urea excretion. Such a decrease we have seen more often than an increase. Probably this is due to renal fatigue and not to renal damage. Even so, it indicates the possibility of harm from overvigorous diuresis. If in the presence of œdema nitrogen output is only moderately increased by diuretics, this should arouse skepticism as to the value of diuretics in patients without œdema in whom their toxic condition indicates retention of toxic substances. What evidence have we that diuretics in any way increase the output of these hypothetical toxic substances possibly nitrogenous in nature? It seems to me that we have very little acceptable evidence for this. Although in cases without obvious œdema after the use of diuretics we do at times get a considerably increased elimination of fluid and salt, to a less degree of nitrogen, we do not know that this benefits the renal condition. The possibility of doing damage with diuretics always has to be kept in mind. Unlike digitalis we have very unsatisfactory signs of real damage from diuretics. If a diuresis does not occur it is probable that a continuation in the use of diuretic drugs will work an injury. If a diuresis does occur we are not sure that this is beneficial except in so far as the removal of fluid makes the patient more comfortable. Possibly our tests of renal function may prove very useful in determining benefit or harm from diuretics. At present the factors concerned in diuresis are far too little known and much study of the action of diuretics in chronic nephritis in man are needed before we are prepared to utilize them rationally in the treatment of nephritis. In such a study it would seem that very little help is

to be expected from experimental pharmacology. The work must be done on man. With our greatly improved methods of clinical observation and particularly with our methods of testing renal function our clinics probably can advance our knowledge of the action and use of diuretics. We must acknowledge frankly the present meagreness of our knowledge of diuretics and approach the problem with both enthusiasm and scientific skepticism. With this attitude I feel that the study of diuretics is an important problem for the clinician to work on. Moreover it gives promise of yielding important results. Certainly almost any definite knowledge of the laws or principles underlying the action of diuretics will be an addition welcome to all clinicians.

So far I have confined my attention to renal structure and to renal function in relation to diet and diuretics. Other important problems in nephritis are concerned with certain results of the disturbed renal function. In this group come hypertension, uræmia, albuminuric retinitis, œdema, dyspnœa, etc. Of them much study has been made and some of them have been discussed by previous Harvey lecturers and I can add relatively little to what they have said already to you.

Graphic methods of studying the cardiovascular apparatus have emphasized the early occurrence of hypertension, cardiac hypertrophy and myocardial disturbance in many cases of nephritis, but they have not thrown much light on the relation between them and the renal lesion. In my own clinic where we have made much use of the electrocardiograph in the study of the cardiac condition of our cases, I have been struck with the great frequency of cardiac changes in cases of chronic nephritis and the very great importance of them as causes of the patients' incapacity. Particularly interesting in this connection have been cases in which hypertension seemed undoubtedly the primary condition, myocardial disturbance secondary and renal insufficiency a negligible feature in the case. Studying the cases we find very slight evidence of impaired renal function and in a few fatal cases the kidneys have been essentially normal as far as glomeruli and tubules were concerned. There was marked chronic passive congestion of the kidney, for these patients have

died from cardiac insufficiency. Arterioles everywhere showed thickening and therein seemed to be the cause of the hypertension. It has seemed that in this type of case myocardial insufficiency was slower in appearance than in patients with renal insufficiency and hypertension. All clinicians are familiar with this type of patient, but it seems to me that they are probably more numerous than we have supposed, because without studying their renal function we have assumed them to have a more extensive renal lesion than they do and so classified them among our cases of chronic nephritis when really they did not belong there. Possibly a more careful study of this group may throw some light on the mechanism of the hypertension in chronic nephritis.

None of our studies have been concerned with the cause of uræmia. Its importance is obvious, but none of our group of workers have possessed the chemical knowledge needed for such a study. The primary problem in uræmia is to know the nature of the toxic substance that causes the uræmic manifestations. Important studies have been made on the problem in other laboratories, but not yet have they advanced to the point of giving us a means of recognizing toxic substances and studying their distribution and effects as a part of the problem of the clinical study of nephritis.

In our cases Woods [26] has made a careful study of the eye grounds to see if any relation between albuminuric retinitis and renal function could be found. It has been claimed by Widal and his co-workers [27] that albuminuric retinitis was closely related to nitrogen retention. To see if this was true the total non-protein nitrogen of the blood was determined in a group of cases and the figures obtained tabulated along with the changes observed in the retina. Naturally since in cases of severe nephritis retinal changes are more common than in mild cases, our cases with high non-protein nitrogen figures showed retinal changes more often than those with low figures. However, there was no relation to be made out between the retinal disturbance and degree of nitrogen accumulation in the blood. To see whether there was any parallelism between the occurrence of retinitis and the amount of various forms of nitrogenous bodies in the blood, deter-

minations of urea, uric acid, creatin, creatinin, ammonia and amino-acid nitrogen were made, but no relationship could be found to exist. It was thought possible that some relation might exist between the amount of non-proteid nitrogen in the spinal fluid and retinal changes, so this was determined, as well as the urea content of the spinal fluid, but here again no relationship was found. It was interesting to see how closely parallel to each other ran the urea values for blood and spinal fluid, there being very slight differences between the two, while for total non-protein nitrogen the amount in spinal fluid lagged very considerably behind that in the blood. Not enough spinal fluid was available for determination of the other nitrogenous bodies which we quantitated in the blood.

The dyspnœa of nephritis is of much interest; often it is a very distressing symptom in our patients. It presents itself in many forms. Very often it is paroxysmal with a proneness to nocturnal attacks. Periodicity commonly is a feature, often of the typical Cheyne-Stokes type. In other cases the dyspnœa is present almost continuously. In a few cases it is of the low deep variety, the air hunger type such as we see in association with diabetic coma. In many patients the dyspnœa in large part is associated with the cardiac insufficiency which is marked as a condition due to the hypertension and myocarditis secondary to the nephritis. In other cases the cardiac element is slight or absent. In our clinic Peabody [28] has studied the relation of the dyspnœa to acidosis, as this has been claimed by some to be the chief factor in the production of the non-cardiac dyspnœa of chronic nephritis. Peabody found that in mild cases of nephritis there was no evidence of acidosis. More advanced cases show an acidosis in the sense that much larger amounts of alkali are required to render the urine alkaline in reaction than is normally the case. Such patients, however, show no decreased CO_2 tension in the alveolar air. It is only in the very advanced cases that acidosis becomes marked enough to cause a decreased CO_2 tension in the alveolar air. The evidence at hand favors acid retention in these cases of nephritis rather than an abnormal formation of acid. The usual type of dyspnœa in nephritis is a periodic breathing of an

irregular shallow type and this will persist after the giving of an alkali has rendered the urine alkaline and restored the CO_2 tension to a higher value. In some cases the dyspnœa is of the "air hunger" type and this may disappear after giving alkali. Acidosis does not seem to be a sole factor in any sense in the usual dyspnœa of nephritics who have no cardiac insufficiency. It probably has some influence, but other factors undoubtedly are active. Very likely variations in the sensitivity of the respiratory centre play an important part. If the respiratory centre is abnormally sensitive then very slight changes in the blood may be effective under these abnormal conditions and both changes in the respiratory centre and in the blood are concerned in the dyspnœa of nephritis.

What I have presented to you this evening is far from a complete account of the very interesting phases of the nephritis problem. As I said in the beginning I have made it fragmentary by limiting my discussion to those phases on which I and my associates have worked. I have purposely refrained from presenting tables, figures or statistics, as such details are so difficult to keep in mind while listening to a paper. They may be found by those specially interested in the individual reports of our work which have been published in current medical journals since 1908 and for which references will be found in this address when printed. If I have succeeded in rekindling any interest in the fascinating problems of nephritis by presenting this account of our work, my purpose has been fully accomplished.

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STUDIES ON INTERMEDIATE CARBOHYDRATE METABOLISM

By DR. R. T. WOODYATT

Otho S. A. Sprague Memorial Institute Laboratory for Clinical Research

INTRODUCTION

IN 1842, F. Bidder observed that the metabolism as a whole comprised two circuits, a greater circuit beginning with the entrance of matter into the body from the outside world and ending with the return of this matter to the outside world; and a lesser laid between the ends of the greater, this lesser circuit beginning and ending within the organism proper. In 1847, Bidder and Schmidt, alluding to this distinction (in their monograph on The Digestive Juices and Metabolism) employ the term "Intermediarer Stoffwechsel" to designate the transformations of matter within the organism proper. This, as Lusk has noted, would seem to have been the origin of the term. Since then it has been used by some in more restricted senses and applied particularly to the purely chemical processes which occur as intermediate steps in the synthesis, destruction or transformations of proteins, fats and carbohydrates or other body substances. According to this usage processes of absorption, secretion and circulation or transport generally would be omitted from the study of intermediate metabolism, but owing to the intimate connections which exist between the physical and the chemical phenomena, the older usage is here preferred.

During the past few years the staff of the Sprague Institute Clinical Laboratory, including E. J. Witzemann, W. D. Sansum and Russell M. Wilder, have carried out detailed studies of certain phases of this general problem with particular reference to the intermediate metabolism of the sugars. The phases studied have been selected with reference to their bearing on certain general views. To facilitate the discussion of this work and in order that it may not appear disconnected, it will be necessary to develop briefly the point of view from which it was undertaken.

It is assumed tacitly that the intermediate metabolism taken as a whole represents multiple separate chemical reactions, or groups of reactions, confined to certain places or media with provisions whereby certain material and energic reaction products separate out of each and are carried among and into others, thereby bringing all of the separate chemical reactions into a sort of dynamic equilibrium. For purposes of study it is convenient to recognize two metabolic spheres: *a sphere of chemical changes* in which molecules are breaking down, rearranging, building up; *and a physical sphere or sphere of transport,* in which intact molecules, singly or in clusters, are in transit between the sites of chemical change. A third or energic sphere may, for the purposes of the moment, be omitted from discussion. The first two spheres imply matter existing in two corresponding *states:* that undergoing chemical changes consisting of smaller particles carrying an excess of positive or negative charges such as *ions and ion-like residues* in general, that showing little chemical reactivity but greater physical motion—consisting in the larger and relatively non-dissociated or *saturated molecules* and of clusters of two or more such molecules, these clusters ranging in size through the gamut of dimensions which characterize colloid particles.

Anatomic counterparts for these spheres are sought in fundamental classes of media which constitute phases in the heterogeneous or colloid structure of protoplasm and body fluids generally, this being merely the application to the study of intermediate metabolism in higher animals of the precept of Bichat to "first isolate the fundamental tissues of which all organs are made up and study each, no matter where it is found, in order finally to understand the several organs in their special characteristics," and in harmony with the viewpoint of the general physiologists made familiar through the work of such writers as Verworn, J. Loeb, Kühne, Weinland, A. P. Matthews, M. H. Fischer, Lillie and others. A tendency in the special literature covering various aspects of the intermediate metabolism in higher animals is still to deal with special organs as things separate and distinct, and in accordance with this tendency the circulating media, especially the blood, might be considered as representative of the physical

and the cells of the chemical sphere. The blood plasma is of course essentially a medium of transport in which chemical reactions are avowedly slow. The work of Michaelis and of McGuigan has shown specifically for glucose that this metabolite exists in the blood in a state of physical solution. The cells, on the other hand, are recognized as the chief sites of active chemical changes. But individual cells possess powers of absorption, secretion and chemical correlation, and even in the blood plasma some chemical changes occur—wherefore it is preferable to regard both plasma and cells as made up of the same fundamental types of media but in different proportions, like the fat and the watery phases in cream and skimmed milk respectively.

If in the study of intermediate metabolism we deal with the fundamental media which constitute the phases of such systems, it is readily understandable how metabolites such as glucose, amino-acids, and others, existing within the body, may be distributed between such phases in definite proportions, how in one type of medium they may exist in a state of simple physical solution, and in another encounter conditions (enzymes, catalysts, or what not), which will favor a high degree of chemical dissociation. Illustration is afforded by the well-known behavior of a fatty acid such as butyric acid, which when added to an emulsion of benzene and water divides itself between the benzene and the water phases in accordance with laws which pertain to the partition of dissolved substances between two partly immiscible solvents. The butyric acid then exists in the benzene in simple physical solution as single molecules in dynamic equilibrium with clusters of two or more molecules—the twos predominating—while in the water the butyric acid exists as single molecules in dynamic equilibrium with hydrogen and butyrate ions. In the water the dissolved substance is sufficiently dissociated to enter into chemical reactions characteristic of acids, such as the turning of ordinary indicators, the liberation of CO_2 from sodium carbonate, etc., but in the benzene such reactions occur if at all only to a very minute extent. The degree of dissociation of the butyric acid in the water phase may of course be greatly influenced by the simultaneous presence there of other substances.

The study of intermediate metabolism resolves itself therefore into a study (1) of the chemical reactions which occur among metabolites when mixed together in different proportions in homogeneous media which favor a high degree of dissociation and chemical reactivity; (2) of the physical or physico-chemical reactions which occur when they are mixed in physical solvents; and (3) of the modifications which occur when the physical and chemical solvents are in contact with one another as phases in an heterogeneous system. For the explanation of reactions of the first type, chemical dissociation and the laws of chemical equilibrium will be of the greatest service, for the second, the laws of molecules, and for the two taken together, the laws of equilibrium in heterogeneous systems. In the final analysis all phenomena of intermediate metabolism must represent the working out of these underlying principles.

METHODS

The recognition of processes proceeding within the organism in accordance with laws of chemical equilibrium must necessarily depend on the successful application of the principles of quantitative chemistry to the study of various biological reactions now known in a purely qualitative sense. Indispensable to the movement in this direction is the development of refined methods of urine, blood and tissue analysis which has received such material advancement in this country through the efforts of Folin, Van Slyke, S. J. Benedict, Shaffer, McCrudden and others. A further necessity consists in methods which will enable us not only to detect but *to create at will within living cells different, definitely related concentrations of selected substances and to maintain these concentrations for periods of time sufficiently long to permit measurements of the rates at which different physiological processes proceed under these conditions.*

When dealing with simpler marine forms definite concentrations of selected substances may be created within the cells by adding known quantities to the sea water surrounding them, and to maintain these concentrations it is only necessary to make the volume of water sufficiently large in proportion to the mass

of the cells employed. In the higher animals the sea water has its counterpart in the circulating media, and to produce a definite concentration of a given substance in the cells it may be made to assume a certain concentration in the blood. But since the ratio of the blood volume to the cell mass is small, in order to maintain a uniform blood concentration, the substance must be added continuously to the blood exactly as fast as it disappears from the blood into or through the cells. Methods of administration in which the substance must be absorbed from the alimentary tract or a subcutaneous, intramuscular, intraperitoneal or other local injection site prior to entering the general circulation, are open to the objection that absorption rates vary with many factors which are not subject to experimental control and but sluggishly and within limits to those which are. An exception should be made of intrapulmonary administrations. Owing to the expanse of absorbing surface presented by the alveoli and the great perfection which has been attained in the development of respiration methods, it is exactly in this field that the most striking progress has been made in the study of quantitative aspects of intermediate metabolism and it is to such methods that the pharmacology of the inhalation anæsthetics owes its distinctive character. But only gaseous and volatile substances are susceptible of administration by inhalation. The majority must be administered in solution and the practical problem then resolves itself into one of ways and means for making direct intravenous injections at predetermined rates and of sustaining them uniformly for long periods of time.

A number of writers have devised apparatus for this purpose, but with the exception of a motor-driven quantitative pump described by Friedenthal, and exhibited at the International Congress of Physiologists at Gröningen, in 1913, none would appear to have embodied the qualifications necessary for work of the type under consideration. Nor are we aware of the existence in the literature of descriptions of experiments which would indicate that intravenous injections have been carried out with any type of apparatus at exact rates for periods of time exceeding three or four hours. During the past two years machines have

Apparatus for Continuous Perfusion.

been developed and used by the staff of our laboratory by means of which direct intravenous injections have been given to dogs and rabbits at predetermined uniform rates for periods of from 4 to 24 hours, and to men for as long as five hours. Earlier models have been demonstrated from time to time, the cut illustrating a newer and more satisfactory instrument. It consists of a graduated glass barrel with a metal piston, the nozzle connecting with a metal block in which is housed a metal valve constructed on the principle of a two-way stop-cock. A "universal" one-twentieth horse-power motor provided with a true and heavy solid metal fly-wheel actuates a worm which turns a driving wheel. The motion of the wheel is imparted by a cam and cam shaft to the valve in front of the glass pump barrel and by a separate motion to the piston itself so that as the piston travels into the barrel, the contents are forced out through one opening, and as it returns, fluid is sucked into the barrel through a tube leading from a graduated reservoir. A link motion permits an adjustment of the length of the piston stroke to correspond with any desired fraction of the length of the pump barrel, and the number of strokes per minute is susceptible of delicate control by the rheostat. Either the coarse or fine adjustment is made without interrupting an experiment. Fluids of any viscosity from that of water to that of citrated blood or 100 per cent. glucose solutions are easily handled. With a uniform source of electrical supply, one may deliver in an hour any chosen volume of fluid from 10 c.c. to 5 litres with an error of only a few tenths of a cubic centimetre. Since the entire action is positive, the rate of delivery may be gauged at any moment by the number of revolutions of the driving wheel as well as from burette readings. This makes possible automatic speed indication. The power and momentum are such that the delivery rate varies inappreciably with changes of intravascular pressure; in fact, if the needle or cannula is intentionally occluded, the rubber tubing which connects the needle and pump will burst before the rate of the pump is greatly affected. All parts which come in contact with the fluid are easily detached for sterilization.

In experiments with dogs the animals have lain on a board

cushioned in blankets with a small cannula inserted into a suitable leg or abdominal vein, under novocain, a catheter in the bladder and no general anæsthesia. Great care has been taken to avoid pain and emotion and the subjects as a rule have remained quiet and frequently dozing throughout the experiments, thereby eliminating non-physiological conditions. Experiments in which such conditions were not attained were considered unreliable. A check on the water balance suggested by Dr. Sansum consists in allowing the animal board to rest constantly on scales so that losses or gains of a few grams in bodyweight become visible through movements of the beam and susceptible of correction if desired, by providing a second pump to discharge water into the delivery tube from the main pump—the second pump idling except when required.

This method has been applied to the study of the effects of different sugars when introduced into the blood stream at different rates, of a number of substances which have come to be regarded as probable or possible intermediates in the metabolism of carbohydrates, fats and protein, and also to the study of a number of salts, both alone and in conjunction with the sugars, etc. We may allude here chiefly to the experiments which bear most directly on the subject of the intermediate carbohydrate metabolism.

EXISTENCE OF GLUCOSE IN TWO STATES OR PHASES

When d-glucose in gram molecular, or 18 per cent. aqueous solutions is administered to normal resting dogs by a peripheral vein at rates below 0.8 Gm. per kilogram of body weight per hour, no glycosuria results even when the administration is continued for hours; but when the rate of administration is increased to 0.9 Gm. per kilogram per hour, glycosuria almost always develops within 30 minutes—the critical rate lying as a rule close to 0.85 Gm. per kilogram per hour. This figure corresponds closely with that fixed by Blumenthal,[1] who employed repeated single injections to determine the intravenous tolerance limit for glucose and also with the figures obtained by Russell Wilder,

[1] Beitr. z. chem. Phys. u. Path., 1905–6, p. 329.

using the present method on human subjects. Thus a man weighing 70 kilograms may receive glucose by vein at a rate of 56 Gm. per hour without glycosuria, which would imply, if the rate were maintained, the introduction of glucose sufficient to liberate 5376 calories per day—a point of some practical interest in the study of intravenous nutrition. But in the present connection the chief points of note are first that the glucose, even when given by peripheral veins without therefore passing through the liver first, does not lead to glycosuria even when the rate of injection is such that the calories would suffice to cover twice over the total heat loss from the body under the conditions of the experiment prior to the beginning of the injection; and second, that with intravenous injections of glucose at rates below those which produce glycosuria, there is no increase in the urinary output of water. In fact, Sansum and Wilder have observed that when a human patient is kept quietly in bed with a uniform hourly supply of water, the hourly voiding of urine may be kept quite uniform, and when such patients receive sub-tolerant doses of glucose by vein in such a way that the total hourly water supply remains unchanged, the rate of urination is, as a rule, diminished during the period of glucose administration rather than increased. This phenomenon of antidiuresis is less striking than that which occurs when glucose is given by mouth or subcutaneously, as described by Lusk, Fischer and Wishart, Allen and others, which suggests that local abstractions of water at the site of administration may be partly but not wholly responsible for the effect.

Now when d-glucose is given at rates above 0.9 Gm. per kilogram per hour, under conditions otherwise the same, glycosuria develops and then diuresis. This effect of intravenous glucose injection has long been known, having been studied especially by Doyon and DuFourt,[2] Pavy,[3] and later several others, including F. D. Allen. When the rate of administration of glucose by vein is maintained uniformly hour after hour, with suitable provisions for the maintenance of the water balance as previously described, the rate of glucose excretion rises from hour to hour in

[2] Jour. de physiol. exper., 1901–3, p. 703; 1901, iii, 703.
[3] Jour. Physiol., 1899–24, p. 479; 1899, xxiv, 479.

a curve, the abruptness of which can be made to vary with factors under experimental control. Ultimately after 3 to 6 or more hours, depending upon circumstances, the rate of glucose excretion becomes virtually constant, the curve then striking a plateau. Once such a level is reached, the rate of the hourly urinary excretion of glucose to the hourly intravenous supply becomes virtually constant. When the injection rate is below 0.85 Gm. per kilo per hour, the ratio of intake to output approaches zero. With an injection rate of 1.8 Gm. per kilo per hour, about 10 per cent. of the intake is likely to be excreted once constancy is obtained. As the rate of injection is increased, the percentage excreted also rises step by step until, when the injection is at the rate of some 3.6 Gm. per kilo per hour, the rate of excretion averages 35 to 40 per cent. of the intake rate. What absolute percentage of the 3.6 Gm. is excreted will depend upon a variety of conditions—notable among which are the water, salt, acid and alkali balances, but especially the water balance. Gilbert and Carnot[4] have already held on the basis of single large injections that the rate of the output to the intake becomes constant at or near 44 per cent. with doses exceeding a certain size, and Kleiner[5] recovered on an average 60 per cent. of the glucose injected in a certain set of experiments. These higher ratios represent comparisons between total quantities of glucose injected and excreted from the beginning to the ending of waves of injection and excretions, respectively. The 35 to 40 per cent. excretions to which we refer are based on comparisons between uniformly maintained injection and elimination velocities enduring for 3 to 12 hours or more, the total quantity of glucose injected per hour being varied by varying the volume of the standard glucose solution injected. Naturally, however, the greater the absolute weight of the glucose administered the greater is the absolute quantity which escapes unchanged in the urine and the greater the volume of water carried with it. As larger and larger volumes of an 18 per cent. glucose solution are injected, a time comes when the water in the solution itself is not sufficient to counterbalance the rate of diuresis. Extra

[4] Compt. rend. Soc. de Biol., 1898–50, p. 330; 1898, l, 330.

[5] Jour. Exp. Med., 1916–23, p. 507; 1916, xxiii, 507.

water is then drawn from the tissues and more water must be superadded from the auxiliary pump if we are to maintain a constant body weight and uniform sugar concentration in the blood and cells. Working with normally nourished healthy animals and 18 to 20 per cent. glucose solutions, and maintaining the water balance at whatever level is found at the start of the experiment, the 35 to 40 per cent. excretion occurs with remarkable frequency. By using more concentrated sugar solutions, allowing the dogs to become partly dehydrated and then maintaining the lower water level, higher percentage excretions of glucose may be obtained. *But whatever absolute percentage of the 3.6 Gm. or more of glucose per kilogram per hour given by vein is excreted in the urine, the same animal under otherwise similar conditions will excrete almost exactly the same percentage of the injected sugar when the rate is 3.6, 4.5, 5.4, 6.3 and 7, or any intermediate number of grams per kilograms per hour.* These experiments indicate that when glucose is made to enter the organism uniformly at a rate exceeding a certain minimum, a definite proportion of it constantly and permanently disappears and the remainder as constantly escapes in the urine unchanged. One fraction suffers a chemical fate, the other a physical fate. Under the experimental conditions described, out of each 10 molecules of glucose which enter the body, we may say roughly 6 suffer a chemical fate and 4 a physical fate, no matter how many are given.

The explanation suggested is that glucose within the body exists in two forms, (*a*) as non-dissociated molecules, (*b*) as unsaturated residues formed by the chemical dissociation of molecules—the two tending to be in equilibrium with one another as is the case with glucose and glucose ions in simple aqueous solutions as shown by the studies of Cohen, A. P. Matthews, Michaelis and Nef. The constant ratios observed are not considered as necessarily indicative of a relationship prevailing between the molecules and ions in any single homogeneous medium, however, but a relationship prevailing in the body, taken as a whole, in which glucose is distributed between the phases of an heterogeneous system in one of which it is highly dissociated, whereas in the other it is dissociated only to a minute degree. The two fractions

are adapted for separate consideration and we may first allude to certain points of interest in the behavior of the non-dissociated glucose molecules, or glucose in the physical sphere.

GLUCOSE IN PHYSICAL SPHERE

When glucose is given by vein to completely depancreatized dogs, the ratio of the injection rate to the output rate approaches unity. The same applies when lactose and many inorganic salts are given in health. In this situation all, or virtually all, of the injected glucose fails of dissociation and we have purely the effects of the molecules. It would not appear, however, that there is any essential difference in the physical effects which are produced by administering glucose to a diabetic at a rate exceeding the tolerance rate by a certain definite margin and by administering glucose to a normal dog at a rate sufficient to cause the same rate of glycosuria, although it has been noticed in a long series of experiments with normal animals that the maximum urinary sugar concentration has been between 8 and 9 per cent., whereas higher percentages than this are known to occur in diabetes melitus (12 to 13 per cent.) and in phorhizin diabetes. In an 8.5 per cent. solution of glucose in pure water, the ratio of glucose molecules to water molecules is roughly 1 to 100. Something less than one hundred would appear to be the minimum number of water molecules which must pass out of the body with one glucose molecule in experiments of the present type. If an unlimited supply of water is available, the urinary sugar concentration is always lower than this. But there is also apparently an upper limit to the number of molecules of water whose passage through the body will be hastened by the presence of glucose molecules. The possibility is therefore presented of fixing the maximum and minimum quantities of water which may accompany a molecule of glucose into the urine, and the question arises as to whether these maximum and minimum glucose water ratios correspond to different hydrates of glucose. In this connection work has been carried out with other substances, such as lactose and inorganic salts in the attempt to establish their diuretic co-efficients with sufficient accuracy to arrange them in a numeri-

cal scale and to ascertain in particular whether the diuretic power of a substance is proportional to the electrical charge on the molecule and the ability of the molecule to hydrate itself. Certainly isosmotic solutions of glucose, lactose, potassium acetate, citrate, and other salts are far from being isodiuretic. Their diuretic power, according to M. H. Fischer, parallels in a general way their ability to make protein gels give up water, and Hofmeister originally attributed the separation of water from protein gels under the influence of salts to the tendency of the salt to hydrate itself. E. J. Witzeman points out a suggestive parallelism between the observed diuretic powers of certain salts and their ability to change the percentages of ethyl-acetate, alcohol, acetic acid, and water in this well-known equilibrium in a manner which has been explained by the Italian chemists to be due to the tying up of water molecules in the form of hydrates of the salts, thereby affecting the equilibrium as though water had been abstracted.

Concerning the *laws of glucose excretion* and the relationships which prevail between the urinary and the blood glucose, the present technic enables one to maintain a constant blood sugar concentration, a constant urinary sugar concentration, or a constant total excretion rate, while other factors are varied. Ambard's laws have been tested in this way and so far found to explain the results obtained.

The most striking effects arising from glucose molecules are connected with the shifting of water. If the glycosuria is maintained at a high rate by a correspondingly high injection rate, and if water is provided to the limit which can be carried away per hour, enormously great volumes of fluid can be passed through the body. A certain dog excreted for 8 consecutive hours an hourly average of 600 c.c. per 10 kilograms of body weight, a rate of diuresis which for a man of average weight would correspond to 4800 c.c. per hour. Attempts have been made to apply this principle in *the treatment of experimental poisonings* with heavy metals, chloral, diphtheria toxins, etc. The results of this histolavage have been highly suggestive in the case of water soluble poisons, but disappointing in the case of heavy metals.

A good cardio-vascular system and a sufficiently permeable kidney are prerequisite for success. *During the first few hours of such a diuresis there is a heavy washing out of chlorides and urea, after which the hourly excretion of these substances falls to a low level.* The impression which would seem to prevail in the literature that intravenous injections of glucose increase protein catabolism is doubtless based on observations of this initial flushing out in experiments which have not been continued long enough to see the end of the process.

If during a high rate of glucose injection the water lost in the urine is not wholly replaced, the animal loses weight, and when the weight loss reaches a certain grade—which will vary somewhat, depending upon the water level at the beginning of the experiment—the animal begins to grow restless and increases the depth and frequency of his respirations. *If at this stage the water deficit is restored by accelerating slightly the rate of the auxiliary pump, these symptoms disappear and it is remarkable how sensitive the organism is to slight variations of the water balance above and below a certain critical level.* After the appearance of some restlessness and hyperpnœa, if the dehydration is permitted to proceed, the animal shakes and shows every sign of having chills, and at about this time or soon after the temperature rises. *By pushing matters it is possible to produce definite rigors and fever as high as 108° F. in dogs, but unless the dehydration is carried too far, the chills, fever, shaking and hyperpnœa all disappear when the bodyweight is brought back to the proper level by adding water.* Exactly similar phenomena may be produced with other diuretics besides glucose, and in the employment of glucose solutions by vein in the clinic for the *intentional dehydration of individuals with glaucomatous eyes,* chills and fever as high as 102° have been seen. Emphasis is laid on the water balance in this connection. The fever can be made to appear and disappear at will by changing the water level.

Under ordinary temperature conditions, heat loss from the body is accomplished in large part through the evaporation of water. When the water available for evaporation is sufficiently

depleted, this system of cooling must become impaired. Clearly the body's supply of water available for evaporation is reduced when a certain quantity of it goes out as urine, but it is not necessary for the water actually to leave the body. It can be bound by sugar or salt molecules or by protein within the body with the effect of retarding evaporation. If we have two saucers of water standing in the air, and add sugar, salt or protein to one, the water in this saucer will evaporate more slowly than the other and the heat loss will therefore be less rapid. The animal organism might be compared with a physical system consisting of solid salt, salt solution and water vapor. This is known as a monovariant system. At any given temperature the concentration of the salt solution (which implies the concentration of water molecules as well as salt molecules) and the pressure of the water vapor, become fixed at definite values. Or, if in such a system the concentration of the salt solution is fixed, the temperature automatically adjusts itself and becomes fixed at a corresponding point in accordance with the phase rule. *The suggestion is, that one fundamental factor in the maintenance of a uniform body temperature is the maintenance of a constant water concentration* within the body and, to be more specific, a constant water concentration in whatever body fluids function in a way comparable to that of the salt solution phase in the system mentioned. Any factor which would change the concentration of water in this phase would then necessarily result in a change of the body temperature. The addition of salts, sugar or other molecules to the blood faster than can be met by a compensatory mechanism must accomplish this effect. This bears directly on the subject of "salt and sugar fever." Febrogenic poisons which change the affinity of colloids for water might have an entirely similar effect and the chills and fever which may follow injections of substances like salvarsan may be susceptible of explanation on the same fundamental basis.

A word may be said in passing of *the clinical value of intravenous injections of glucose at diuretic rates with intentional dehydrations of the body.* It might be supposed *a priori* that this method would have value in the reduction of certain forms of

œdema, fluid accumulations in body cavities, etc., and cautious experiments have been made in a variety of clinical conditions to test this possibility. In cardiac œdemas the procedure would appear to be irrational and injurious. When glucose is injected into the blood, it passes into the tissues with great rapidity. Meltzer and Kleiner have demonstrated how quickly glucose leaves the blood stream following its introduction into the veins even after death. Once in the tissues in increased concentration glucose molecules cause there a shrinkage of the colloids with separation of water, explainable perhaps on the basis suggested by Hofmeister that the glucose molecules hydrate themselves at the expense of the colloids. But however the effect may be explained, it is, as M. H. Fischer has demonstrated, a phenomenon quite analogous to that which we see when glucose is added in sufficient concentrations to protein gels *in vitro.* Before this water, which is freed from the colloid combinations to become a glucose solution, can leave the body, it must first enter the blood stream and be transported to the kidneys. A great increase in blood volume and blood pressure results. The water which the tired and flagging heart has given the tissues to hold for it while it rests, is again saddled upon it. There may be some increased diuresis, but only at the cost of increased cardiac effort with no compensatory advantage. The same general principles should apply to the use of any intravenously administered diuretics such as salts, whose diuretic effects are ascribable to the same basic mechanism. Such methods are also contra-indicated, according to our observations, when the kidney is so badly and irreversibly changed that even though the heart is strong the kidney presents an insurmountable barrier in the passage of the water separated from the tissues by the sugar. On the other hand, with individuals having adequate cardio-renal mechanisms and localized œdemas, the situation is different. In a certain case of glaucoma we have reduced the intra-ocular tension three times from the neighborhod of 57 to within normal limits inside of an hour and a half, an effect strictly analogous to the spontaneous production of the "soft eyeball" of diabetes by the same dehydrating effect of dissociated glucose molecules.

Riessman has recently studied this condition from the clinical standpoint. Such methods may also be used successfully to start diuresis in certain cases of acute anuria in which the kidneys are strangled by swelling under the capsules—the same type of cases in which decapsulation may be followed by a restoration of function. In such cases the beneficial effects lasting after the injection has ended would seem to be due, as Fischer already has held, to the relief of pressure, the coincident re-establishment of the oxygen supply caused by improvement of the blood flow through the part and the resultant diminution of asphyxial acid accumulations.

THE CHEMICAL PHASE

We may now return to the consideration of the fraction of glucose which during constant intravenous injections at high rates disappears and suffers chemical changes. Metabolism experiments carried out in conjunction with Dr. Walter M. Boothby and Miss Irene Sandiford in the respiration laboratory of the Peter Bent Brigham Hospital, Boston, for the privilege of which we take pleasure in thanking Dr. Harvey Cushing, showed that in dogs receiving 3.6 Gm. of glucose per kilogram per hour—of which about 2.4 Gm. disappeared—not over .6 Gm. gave evidence of actual oxidation. The non-protein respiratory quotient remained close to 1.0 and the assumption was that most of the remaining 1.8 Gm. was polymerized under the conditions of the experiments. By feeding experiments Dr. Lusk had shown that the administration of increasing quantities of glucose caused increases of the total metabolism up to a certain point, after which the feeding of more sugar caused no further increase. The question arises as to whether this indicates a limit to the rate at which glucose can be absorbed, or, as he suggests, an actual upper limit to the amount which can be oxidized no matter how great a sugar concentration is produced in the cells. Although the rate of glucose oxidation indicated by the data of Boothby and Sandiford is somewhat above that obtained by Lusk and by Grafe after feedings of glucose, the discrepancy would seem remarkably slight when it is recalled that glucose when introduced into the

blood at the rate of 3.6 Gm. per kilo per hour in 18 per cent. solution may produce and maintain a .55 to .66 per cent. blood-sugar concentration with a steady and intense glycosuria of 12 to 14 Gm. per 10 kilograms hourly, whereas glucose given by mouth can only cause an hyperglycæmia in the peripheral blood a trifle above the normal threshold value. This would support Dr. Lusk's interpretation (although further metabolism studies are needed) on animals in which the cell and blood sugar concentrations are maintained at levels higher than can be attained by absorption methods of administration and higher than these just described. Until such studies are made, discussions of possible numerical relationships between the fractions of glucose which are oxidized, reduced and polymerized respectively, during the maintenance of different uniform blood sugar concentrations under standardized normal conditions—as well as the factors which might be expected to change such relationships—would be largely speculative. It would appear, however, that normally the rate of glucose oxidation varies within a comparatively small range, while the relationships between the rate at which glucose is precipitated in the form of glycogen and the rate at which it is excreted unchanged, may be more nearly reciprocal. The studies of McLeod bear interestingly on these points.

On questions of the mechanism of glucose polymerization, reduction and oxidation, and the intermediate steps which occur in these processes, a vast amount of study has been bestowed by many workers, and particularly on the question of oxidation. For various reasons it has been inferred that the breakdown of glucose in the body leads to the formation of a chain of smaller molecules of more or less stable and well-defined substances, such, for example, as glyceric aldehyde, lactic acid, pyruvic acid, methylglyoxal, etc. With the exception of lactic acid, which under asphyxial conditions may accumulate to the extent of 0.5 per cent. and more in the muscles and to smaller degrees in other tissues, few if any of these supposed intermediates have been demonstrated within normal tissues or blood, nor has there been any direct method of proving whether or not a suspected substance is actually formed during the oxidation of glucose in the body.

In general, I believe we should not expect that any intermediate which might be formed during the normal oxidation of glucose to carbon dioxide and water will ever be found in appreciable quantities in the blood plasma or urine. The blood plasma is made up, as stated before, almost wholly of a non-ionizing or physical solvent whose principal function it is to transport molecules of food to the various sites of chemical change and to carry molecules of end-products from those sites. An end-product of one reaction mixture may indeed be food for another, in which case it might be referred to as a physical intermediate or an intermediate in a polyphase reaction. Well-defined molecular substances such as glycine, alanine, or glucose itself are examples of such physical intermediates in the building up, oxidative breakdown or transformation of the more complex body substances such as proteins, polypeptids, glycogen and polysaccharides respectively. Many such reactions as the formation of glycogen from protein are scarcely capable of duplication *in vitro* in any one homogeneous medium. Hence for their fulfilment substance must pass from one chemical reaction mixture to another through what we have called the physical sphere, or sphere of transport, in which a molecular state of matter prevails. But the oxidation of glucose to carbon dioxide and water can be carried out easily in a simple aqueous solution of any ordinary alkali, such as caustic soda, by means of air, hydrogen peroxide or other oxidizing agent, and there is no necessity for assuming that in the oxidation of glucose *in vivo* certain intermediate products are formed which cannot be oxidized further at the site of their origin, but must diffuse out into the physical phase in the form of molecules for transport elsewhere, and so be found in the blood plasma and similar media. In the normal oxidation of glucose in the body the chief substances of this type are carbon dioxide and water. All intermediate steps between glucose and carbon dioxide and water might be expected to take place in one type of medium—a medium in which the prevailing state of matter is ionic or ion-like. Ions as such are not known to be capable of diffusing out of the medium on which their existence depends. If molecules were formed in such a medium and failed to undergo a high degree of dissociation

they should accumulate there in considerable concentration and so necessarily diffuse out into the physical phase. They would then be end-products of the particular reaction, not true chemical intermediates. The blood plasma as a fluid consisting predominantly of a physical type of media should then contain such products in demonstrable quantities.

This hypothesis can be subjected to experimental test as follows: As stated above, glucose can be made to oxidize in the body at the rate of 0.6 Gm. per kilogram per hour. Glucose burning at this rate in any specified way would necessarily produce its ashes or intermediates at a definitely calculable rate. We may take any proposed scheme of glucose oxidation, figure the rate at which each intermediate would have to be formed according to this scheme, and then administer each of these intermediates by vein at this rate and see whether or not the effects are compatible or incompatible with the hypothesis. It has been proposed that in the normal oxidative breakdown of glucose in the body the great preponderance of the 6 carbon glucose molecules first split into two molecules of the three carbon sugar glyceric aldehyde. This implies that 0.6 Gm. of glucose burning per hour per kilogram of body weight introduces into the body .6 Gm. of this triose per hour. But experiments show that glyceric aldehyde administered at such a rate is decidedly and characteristically toxic, even fatally so. Even at one-sixth of this rate glyceric aldehyde injections cause unchanged glyceric aldehyde to appear in the urine. Neither of these effects is seen when glucose itself is given at any rate up to 7.2 Gm. per kilogram per hour. Therefore we may say that if any molecules of glyceric aldehyde are formed in the oxidative breakdown of glucose the rate of their formation is so small that the glyceric aldehyde concentration produced in the tissues is below that which results from administering glyceric aldehyde at only one-sixth of the rate at which Embden's hypothesis would demand. Recent experiments of a different nature by von Fürth have also cast doubt upon the validity of the idea that glyceric aldehyde can constitute a chief normal intermediate in the glucose catabolism. Quite analogous discrepancies are found when in place

of glyceric aldehyde we substitute lactic acid, pyruvic acid and each of several other molecular substances which have come to be considered as probable intermediates, and the conclusion would seem necessary that none of these substances is a chief intermediate in the commonly accepted sense. No suggestion is made that these substances may not, however, be molecular forms very closely related to the true intermediate substances. They may represent, as Otto Neubauer put it, the nearest separable ("fassbare") products.

Some relaxation of the stiffness of our conceptions of the oxidative breakdown of glucose might be desired. Instead of conceiving well-defined molecular substances as obligatory forms which must be passed through in succession like water flowing from one fixed basin to another in a fountain, we might rather think of the glucose catabolism as a swiftly running, natural river, in the main channels of which there are no forms more fixed than the changing eddies. Molecules might then be conceived as the quieter bayous and backsets away from the principal channels.

URIC ACID IN ITS RELATIONS TO METABOLISM*

PROF. STANLEY R. BENEDICT
Cornell University

ONE hundred and forty years ago Scheele[1] first isolated uric acid from urine and from urinary calculi. He showed that the substance was acid in properties by dissolving it in lime water and reprecipitating it with acid. Twelve years later Pearson[2] found uric acid in the tophi of gout, thus laying the foundation of Garrod's theory of this disease, which was published half a century later.

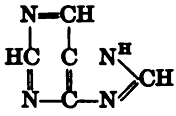
Scheele and Fourcroy[3] and others studied the chemical properties of uric acid in a rudimentary way, but the work of Liebig and Wohler,[4] published in 1838, gives the first account of carefully conducted experiments in this field. These investigators isolated and studied practically all of the oxidation products of uric acid which are known to this day. Perhaps the most important portion of their work from the biological standpoint was the demonstration that allantoin, which had been previously isolated from the amniotic fluid of the cow, could be obtained by the oxidation of uric acid. Subsequently Moritz[5] showed that suitable oxidizing agents may convert uric acid into oxalic acid and urea, both well-known biological products.

Among the numerous papers dealing with the chemistry of uric acid, that of Medicus,[6] published in 1875, is of peculiar interest. In this paper Medicus proposed for uric acid the formula which seven years later Emil Fischer showed to be correct—a beautiful example of a chemical prophecy, if we may use that term.

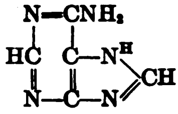
Uric acid is the most highly oxidized member of a group of compounds termed "purines," the most important representatives of which, adenine, guanine, hypoxanthine, xanthine, theobromine, caffeine, and uric acid, are represented in Table I.

* Delivered April 8, 1916.

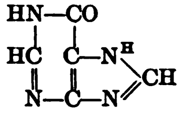
TABLE I



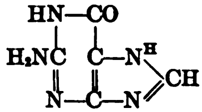
Purine



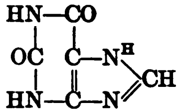
Adenine
6-aminopurine



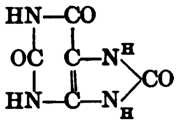
Hypoxanthine
6-oxypurine



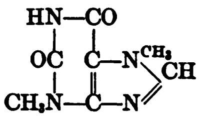
Guanine
2-amino-6-oxypurine



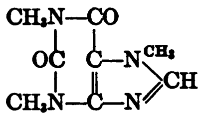
Xanthine
2-6-dioxypurine



Uric Acid
2-6-8-trioxypurine



Theobromine
3-7-dimethylxanthine



Caffeine
1-3-7-trimethylxanthine

In coming to consider the physiology of uric acid, we may note in passing its remarkably wide distribution in the animal kingdom. It is found in nearly every animal form, from insects to man. Garrod found it in all insects examined except spiders, which excrete guanine in place of uric acid.[7] Hopkins[8] found that the powdery scales on the wings of butterflies consist of uric acid. Birds and reptiles excrete large quantities of the substance, which has also been found in the blood and urine of practically all mammals examined.

We shall now consider the substances from which uric acid may be formed in the animal body and the mechanism of such formation. In the days of Liebig the current view of the origin of uric acid was that it represented a partial oxidation product of all protein materials. If the body had a rich oxygen supply it was believed that nearly all the uric acid formed was further oxidized, while if the oxygen supply was deficient, uric acid was destroyed in much smaller proportion, and gout or some similar condition was likely to develop. This view was accepted for a long time and it was not until the early eighties that Kossel[9] laid the foundation stones of our present theories of uric acid formation. A great many men have aided in working out the problem, but we must mention especially the names of Horbaczewski, Kruger and Schmidt, Burian, Schittenhelm, Jones and Spitzer on the biological side, and of von Baeyer, Emil Fischer and P. A. Levene on the chemical side. Von Baeyer and Fischer worked out the formulæ of the purines and Levene solved the question of the structure of the nucleic acids.

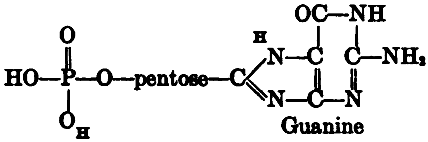
Kossel's work showed that certain purines were to be found among the decomposition products of the widely distributed nucleoproteins. Fischer had already shown the close chemical relationship of these purine bodies to uric acid and Kossel at once suggested that they might constitute the source of uric acid in the animal body. A number of experimenters, on account of unsuitable technic, both as to analytical methods and animals employed, failed to find experimental proof for Kossel's suggestion. Finally, however, Horbaczewski,[10] in a series of beautiful experiments, gave the first demonstration that animal tissues can transform the purines of nuclear material into uric acid. By digesting a tissue rich in nucleic acid, such as the spleen, with blood, he obtained the purine bases hypoxanthine and xanthine in the absence of oxygen, or uric acid when oxygen was present. Horbaczewski also found that feeding of spleen or other glands to either man or the rabbit was followed by an increase in the uric acid eliminated in the urine.

Following Horbaczewski's work, studies were undertaken by various investigators to find the mechanism by which uric acid

is formed from nucleic acids in the body. In this field the researches of Schittenhelm and especially of Jones stand out preëminently.

Levene has shown that nucleic acids represent a grouping together of several simple so-called nucleotides. A typical example of a simple nucleotide is guanylic acid (Table II) in which

TABLE II



Guanylic Acid

guanine is linked to phosphoric acid by means of a pentose radicle. It is from the purine portion of the nucleic acids that uric acid has its chief origin in mammals. The process of the transformation is somewhat complex. The work of Schittenhelm and of Jones has shown that there are at least nine separate enzymes which may be concerned in it. We may briefly indicate the paths for the transformation of the purines of nucleic acid into uric acid as follows: The initial splitting may be into mononucleotides, such as guanylic acid. An enzyme capable of effecting this splitting probably occurs in yeast and has been found in the pancreas of some species. A second type of nuclease, termed "phosphonuclease," instead of splitting the molecule into mononucleotides, will split off phosphoric acid either from mono- or polynucleotides, leaving the purine base joined to the carbohydrate as in guanosine. A third type of enzyme splits off purine bases from the original molecule, leaving the phosphoric acid and carbohydrate radicles joined together.

So far as the purine portion of the molecule is concerned, the initial splitting by nuclease may therefore give rise to either free purine (as guanine) or to purine joined to carbohydrate, as in guanosine. The guanine is converted into uric acid through two steps. First the enzyme guanase splits off NH_2 and replaces it by an oxygen atom, giving xanthine. This in turn is acted upon by xanthinoxidase which converts it into uric acid through the addition of an atom of oxygen. The transformation of the

purine in guanosine may take place in either of two ways. The base may be set free by a hydrolytic enzyme and be then converted into uric acid, as we have just indicated, or the NH_2 may be replaced while the purine is still attached to carbohydrate, giving rise to xanthosine, which is then split into its two components. The xanthine is then directly oxidized to uric acid. Where the original purine is adenine instead of guanine the steps involved will be exactly analogous, with the additional step of oxidation of the hypoxanthine formed by deamidation of the adenine to xanthine.

The enzymes concerned in these transformations show widely different distribution for different species. For the most part they are found in the liver, spleen, pancreas and thymus. It is worthy of note that adenase, the enzyme which transforms adenine into hypoxanthine, cannot be demonstrated for human tissues *in vitro*. Guanase is lacking in the pig, which is subject to guanine gout.[11]

In mammals the preformed purines of nucleic acid constitute the chief if not the only source of uric acid. In birds and reptiles this is not the case. Between 60 and 70 per cent. of the total nitrogen of the urine in these species is eliminated in the form of uric acid, and it is obvious that, in such animals, there must be sources of uric acid other than the nucleic acids. Minkowski [12] has made a special study of this problem in geese and has shown that in this species uric acid can be synthesized from the very simple compound ammonium sarcolactate. In geese with extirpated livers uric acid almost wholly disappears from the urine, its place being taken by ammonia and sarcolactic acid. After Minkowski's work Kowalewski and Salaskin [13] furnished final corroboration of his results by showing that if blood containing ammonium lactate be perfused through an isolated goose liver it is changed into uric acid. The nitrogen of urea and of various amino-acids has also been shown to be converted into uric acid under such conditions.

There is thus a twofold source of uric acid in birds: *viz.*, from the nucleic acids and from purine-free nitrogenous compounds. The question naturally arises as to whether there may be such a twofold origin of uric acid in mammals.

For the young animal this question is easily answered in the affirmative. A growing animal on a milk diet, for instance, receives no nuclein or other purine-containing substance in the food, and yet eliminates uric acid and other purines in the urine and synthesizes a large amount of nuclear material for the body cells. Hence, such an animal must have the power of synthesis of purines from non-purine materials. In the case of adult animals the question is not so readily answered. The food here constantly contains appreciable quantities of purines, and the impression has prevailed very widely that these food-purines constitute the sole source of the purine bodies catabolized in the adult mammal. At least, definite proof has been lacking that this is not the case, since the ingestion of non-purine food substances of widely varying structure has not led to any specific increase in the uric acid eliminated. We shall return to this question later. In the meantime we may consider some of the factors which appear to influence uric acid formation, especially in man.

Burian and Schur [14] were the first to call attention to the fact that under ordinary conditions of diet there must be a double source of uric acid in the adult mammal; *viz.*, the nuclear or purine material ingested in the food, and the nuclear material catabolized by the body from its own tissues. To the uric acid formed from the food purines Burian and Schur gave the name "exogenous" uric acid, indicating its outside origin, while the uric acid formed from the body's own store of nuclear material was termed "endogenous" uric acid. In accordance with this view it is found that when a man changes from an ordinary mixed diet to one which contains no nuclear or purine material the uric acid eliminated falls markedly in amount but does not by any means wholly disappear. That portion which does disappear is obviously exogenous in origin; that which persists is endogenous.

Studies on exogenous uric acid formation have been carried out by a number of observers. Burian and Schur, Minkowski, Kruger and Schmid, and more recently Mendel and Lyman, in an interesting and extensive investigation, have reported valuable observations upon the conversion of free purine bases into uric

acid in man. Mendel and Lyman [15] found that about 60 per cent. of ingested hypoxanthine, 50 per cent. of xanthine, 19–30 per cent. of guanine, and 30–37 per cent. of adenine were eliminated as uric acid.

Although uric acid elimination may be much increased after the ingestion of glandular tissues, such as thymus, only a small fraction of the purine nitrogen reappears in the urine as uric acid. Givens and Hunter [16] have recently reported similar results following ingestion of pure sodium nucleate. What becomes of the balance of the purine nitrogen in these experiments is unknown. The suggestion that it undergoes bacterial decomposition in the intestinal tract is not very satisfactory, in view of the very high percentage which is usually lost.

We may summarize the findings concerning exogenous uric acid by saying that it seems definitely proven that purines, ingested either as free base or in the form of nucleic acid, are at least partially converted into uric acid. The possibility that the purines may have some other path of catabolism than through uric acid has not been excluded.

In a discussion of endogenous uric acid I am reminded of a statement made by Professor Mendel, in his lecture before the Harvey Society ten years ago, upon the subject of uric acid formation. In summarizing some problems for the future Professor Mendel said, "We assuredly need to know more about the origin and significance of endogenous uric acid." It is, perhaps, a sad commentary upon progress that after these ten years it seems necessary to alter Professor Mendel's statement and say, "We want assuredly to know *something* about the origin and significance of endogenous uric acid." The subject is almost chaos, and we have neither time nor inclination to enter into a discussion of the conflicting results of various investigators. There are, however, a few facts which may bear scrutiny. The lowest possible level of uric acid excretion seems to be reached upon a diet very poor in nitrogen but which yields calories enough to protect most of the body's own tissues. Folin called attention to this fact some ten years ago, and it has recently been corroborated in an interesting paper by Ringer and collaborators.

If the nitrogen intake be slightly higher than the minimum level obtained with a starch and cream diet, there is a slightly greater output of uric acid. If the protein intake be again raised there is no increase in the uric acid output, at least until the nitrogen ingested is well above that contained in our ordinary diets. With a protein intake of 30 or 40 grams of nitrogen per day the uric acid elimination will again show a marked rise. This latter point is shown very beautifully in a recent paper by Taylor and Rose.[17] It is probable that the extra uric acid eliminated in such an experiment is not formed from the protein ingested but rather represents body nuclear material broken down under the influence of the forced protein feeding, a possibility suggested by Taylor and others, or perhaps the excessive activity of the digestive glands may be an important factor in such cases.

Concerning the organs or tissues which may be directly responsible for the production of uric acid, very little can be said. There are only two theories which deserve mention in this connection. One of these, suggested by Burian, assumes that the free hypoxanthine of muscle tissue is converted into uric acid and that such conversion is increased by muscular work. Burian's experiments seemed to support this view, but Siven [18] was not able to reach definite conclusions as a result of his work in this connection. This latter investigator found that uric acid elimination was higher during the waking hours than during the night, but was unable to demonstrate an effect of muscular work. In spite of a recent paper by Ringer [19] and his co-workers which contains a single experiment supporting Burian's view, we must conclude that at present nothing can be definitely stated concerning a possible influence of muscular work upon uric acid elimination.

The view that glandular activity, especially that of the digestive glands, is responsible for the formation of some or all of the endogenous uric acid, was proposed many years ago by Mares, and has been the subject of much work and more controversy. Siven has opposed the theory, while Hopkins and Hope,[20] Smetanka [21] and others have offered evidence in its favor. Mendel and Stehl [22] recently reported further experiments which favor the view that the digestive glands may contribute to uric

acid formation. Their results, like those of Smetanka, seem to show a definite rise in uric acid elimination following an ingestion of purine-free food (protein or fat plus carbohydrate). The hourly variations in uric acid elimination during fasting are so wide in the work reported by Mendel and Stehl that one must be cautious in drawing conclusions from their experiments. The Folin-Shaffer method for uric acid determinations which they used is hardly suitable for determining *hourly* uric acid elimination. A repetition of their work employing the micro-method of analysis for uric acid is urgently called for. Nevertheless, we may say tentatively that the balance of evidence is in favor of the view that the digestive glands may contribute to uric acid formation.

The urine of practically all mammals except man and the anthropoid ape contains an oxidation product of uric acid, allantoin, in considerable quantity and the uric acid is correspondingly diminished or almost absent. Furthermore, various tissues of these species which eliminate large amounts of allantoin in the urine have been found to have the power of destroying uric acid *in vitro* with a corresponding formation of allantoin. The tissues of man and the higher apes do not exhibit this power of uric acid destruction and only minimal traces of allantoin occur in the urine of these species. Hence, it is concluded that uric acid is certainly not the chief end-product of purine metabolism in any mammal so far studied except man and his first cousin. By determining the ratio of allantoin nitrogen to uric acid nitrogen plus allantoin nitrogen in the urine, Hunter and Givens have calculated what they call the "uricolytic index," or power of uric acid destruction for various species. This index shows uric acid destruction varying from 79 to 98 per cent. in widely separated species of the lower mammals, while in man and the chimpanzee the index would be approximately zero.

Schittenhelm has long contended that man possesses quite a marked power of uric acid destruction in spite of the fact that Wiechowski has been able to recover in the urine nearly all of the uric acid injected subcutaneously into man and in spite of the further fact that no power of destruction of uric acid can be

demonstrated in human tissues postmortem. While the evidence which Schittenhelm offers in favor of uric acid destruction by man is far from convincing and has been recently partly refuted in a paper by Fine,[23] it is also true that the evidence against this view is not final. It has been found, as will be mentioned later, that certain free purine bases behave differently in the body from the same purines when given in combination in nucleic acid, and if it should be shown that uric acid or the aminopurines may go to other products than allantoin, then Schittenhelm's view may have a certain degree of plausibility. If, for instance, uric acid as formed in the organism were in a combination, it is possible that such uric acid might be destroyed, although free uric acid, either injected subcutaneously or mixed with tissue postmortem, might escape such destruction. As we shall see later, there are some facts which might lend support to these suggestions.

The study of uric acid metabolism has been much hampered by the fact that no animal has been available for experimental purposes in which uric acid is an end-product of metabolism or in which the essential features of human purine metabolism appear to be duplicated. It is therefore of interest to know that recently an animal has been found which appears to fulfil these conditions, and in which it seems probable that we shall find some of the missing links between the purine metabolism of man and other mammals.

The animal in question is the Dalmatian breed of dog. This breed is commonly known as the coach or carriage dog and is characterized by its spotted or mottled skin. A dog of this breed, studied in this laboratory a little over a year ago, showed a very peculiar anomaly of uric acid metabolism. Simple addition of hydrochloric acid to his urine was followed by the immediate formation of a heavy amorphous precipitate. On standing a short time this precipitate assumed the characteristic form of pigmented uric acid crystals. Isolation and analysis of the purified crystals showed them to consist of uric acid. The substance has repeatedly been isolated in large quantities from this urine and analyzed. An examination of the urine showed that this ani-

mal upon a purine-free diet was eliminating almost as much uric acid per day as would an average-sized man on a similar diet, and this in spite of the fact that the dog weighed only about ten kilograms. Furthermore, uric acid injected under the skin was eliminated quantitatively as such in the urine. These findings are the more noteworthy because the ordinary dog shows a destruction of from 98 to 100 per cent. of uric acid either subcutaneously introduced or that formed in metabolism. It was at first supposed that the anomaly of purine metabolism existed only in the one individual, but further examination has shown that it is probably a peculiarity of the breed of Dalmatians. We have examined five animals, of more or less pure blood, and in four of them have found a very high uric acid elimination. The one exception was obviously not of very pure breed.

It may be noted in passing that it seems difficult to find any analogy for the perversion of purine metabolism in the Dalmatian. Conditions such as cystinuria or pentosuria, which are found in some human individuals, would be closest to it, but in such cases the peculiarity is individual and not racial.

In connection with the experiments reported upon the Dalmatian dog, I desire to express my indebtedness to Mr. Emil Osterberg of our laboratory, whose constant and most efficient help has been invaluable.

Table III shows the general metabolism of our Dalmatian as represented by the urinary findings upon purine-free diets with varying nitrogen content. It will be noted that the uric acid output is not increased following a four-fold increase in the nitrogen content of the food. The allantoin (determined by Wiekowski's method) shows, on the contrary, a distinct rise following the increased nitrogen ingestion.

For a period of nearly a year the animal has been upon a purine-free diet and during nearly all this time the uric acid elimination has been determined daily. As a result of this study we can definitely conclude that the adult mammal can synthesize purine from non-purine material. During the period of observation the dog maintained a constant body weight and eliminated a total of more than 100 grams of uric acid. Not 10 per cent. of

TABLE III

Total N grams	Creatinine N grams	Uric acid N grams	Allantoin N grams	Remarks
4.8	0.103	0.123	.....	Diet contains 5.9 grams total N.
5.0	0.106	0.119	0.057	
4.9	0.103	0.126	0.064	
4.9	0.105	0.121	0.061	
5.1	0.104	0.120	0.051	
16.6	0.106	0.117	0.037	Diet contains 24.07 grams total N.
18.4	0.110	0.119	0.107	
19.8	0.109	0.128	0.103	
17.5	0.111	0.123	0.102	
15.2	0.104	0.116	0.083	
5.4	0.104	0.154	0.073	Diet contains 2.03 grams total N.
4.1	0.100	0.139	.....	
3.4	0.100	0.139	0.068	
3.0	0.094	0.141	0.054	
2.8	0.091	0.139	0.059	
2.7	0.084	0.140	0.057	

this quantity of uric acid could have come from the preformed purines of the animal's tissues. So far as I am aware, this experiment is the first which definitely shows that an adult mammal can synthesize the purine nucleus.

The influence of caffeine ingestion upon uric acid elimination is a question which has received much study. A number of investigators have failed to note any increase in uric acid output after the feeding of caffeine to man.[24] Taylor[25] obtained the opposite result, but the general conclusion has been that caffeine ingestion in man does not lead to increased uric acid formation.

In Table IV are recorded two experiments upon our Dalmatian to determine the effect of caffeine given subcutaneously. A daily dose of 100 milligrams of the drug is followed by a slight decrease in the uric acid output. Although the variation is not marked, we have obtained it in each of three similar experiments. With a larger dose (200 milligrams daily) of caffeine there is scarcely any perceptible effect upon the uric acid output, but there is a very notable retention of nitrogen during this period.

In Table V is recorded an experiment to study the effect of caffeine ingestion upon the uric acid output in man. The micromethod of analysis was employed for the uric acid determination, a method which we have found to be highly accurate. The subject was placed upon a purine-free diet, which was kept approximately constant but which was not weighed. During the preliminary period five cups of a caffeine-free (Kaffee Hag) were ingested daily. During the caffeine period the diet was just the same, but to each of the five cups of coffee taken were added 200 milligrams of caffeine, making a total of 1 gram of caffeine per day. The uric acid figures of the urine showed a slight but definite and progressive increase during the caffeine period, which increase was still somewhat apparent for two days after the caffeine intake was stopped. This experiment was so carefully conducted and the results are so clearcut that I believe we are justified in concluding from it that caffeine may lead to increased uric acid formation in man, and furthermore, as evidenced in Tables III and IV, that it may lead to some nitrogen retention. Indeed, on the basis of our experiments, I am somewhat skeptical as to whether caffeine, even in small doses, is quite so innocuous a substance as we have assumed it to be.

In Table No. VI experiments are reported showing the effect of the addition of nuclear material in the form of thymus gland to the diet of the Dalmatian. It will be noted that after the thymus ingestion the increase in the uric acid eliminated is not nearly so great as the increase of allantoin. Thus, on the purine-free diet the uric acid nitrogen is more than double that of the allantoin, while after the thymus ingestion the increase in uric acid nitrogen eliminated is only about one-half the increase to be found in the allantoin nitrogen. These results might be taken to indicate that exogenous nuclear material undergoes catabolism along different lines from that of the endogenous purine-containing material. The question involved will require further work before any definite conclusion can be drawn. This is especially evident when we note (as shown in Table VI) that uric acid administered subcutaneously is followed by a marked increase in the allantoin output, and this in spite of the fact that the uric

TABLE IV

Total N grams	Uric acid N grams	Allantoin N grams	Remarks
5.37	0.129		
5.65	0.126	0.213	
6.07	0.128		
5.52	0.125		100 mgm. caffeine.
5.41	0.113	0.192	100 mgm. caffeine.
5.23	0.116		100 mgm. caffeine.
5.13	0.115		
5.20	0.128		
5.34	0.127		
5.31	0.128		
4.82	0.136	.....	200 mgm. caffeine.
4.80	0.139	.....	200 mgm. caffeine.
4.60	0.127	.....	200 mgm. caffeine.
4.22	0.123	.....	200 mgm. caffeine.
4.22	0.128	.....	200 mgm. caffeine.
4.51	0.125		
4.72	0.124		
4.90	0.122		
4.96	0.127		

TABLE V.—SUBJECT E. O.

Volume c.c.	Total N grams	Creatinine N grams	Uric acid N grams	Remarks
1300	12.60	0.495	0.540	
1290	13.12	0.486	0.522	
1300	13.74	0.508	0.540	
1240	10.76	0.504	0.558	1 gram caffeine.
1640	10.42	0.484	0.564	1 gram caffeine.
1210	8.03	0.519	0.642	1 gram caffeine.
1080	10.74	0.531	0.702	1 gram caffeine.
1200	9.05	0.543	0.636	
1100	13.53	0.531	0.600	
1400	12.51	0.502	0.546	

acid is recovered quantitatively as such in the urine. It seems probable that uric acid and allantoin are inter-related in metabolism in other ways than have been heretofore assumed.

We finally turn to what may be regarded as essentially the

TABLE VI

Total N grams	Urea + ammonia N grams	Uric acid N grams	Allantoin N grams	Increase in uric acid N grams	Increase in allantoin N grams	Remarks
4.37	3.86	0.120	0.050	.....	.....	Normal diet.
4.54	4.03	0.125	0.050			
4.82	4.36	0.127	0.050			
6.48	5.87	0.177	0.210			Same diet+100 grams thymus daily=0.32 grams purine N.
6.86	6.15	0.197	0.210	0.223	0.480	
7.30	6.58	0.200	0.210			
5.75	5.27	0.129	0.058	.....	.....	Normal diet.
5.54	5.08	0.132	0.058			
5.27	4.84	0.137	0.058			
5.18	4.50	0.290	0.115	.....	.....	Same diet+500 mgm. of uric acid subcutaneously.
5.13	4.47	0.299	0.142	.....	.....	
5.00	4.30	0.305	0.133			
5.02	4.54	0.124	0.066			

most modern field of uric acid research, the investigations of uric acid in blood. This field of work will be irrevocably associated with the name of Otto Folin. For many years Folin, more than any other man either here or abroad, has been a maker of high-grade tools for the biochemist. In methods of uric acid research he has recorded another notable achievement. Before the Folin method was available it was questionable whether uric acid existed at all in normal human blood. Quantities up to 300 cubic centimetres were necessary for even approximately accurate results with bloods known to contain an excess of uric acid. Now we are able to determine uric acid with accuracy in twenty or even in ten cubic centimetres of normal human blood. The method is equally applicable to the blood of other species.

The finding of uric acid in pathological human blood dates back to the work of Garrod in 1848. With a truly outrageously inadequate method of analysis this brilliant worker was able to obtain results of permanent value and to demonstrate for the first time that there is an accumulation of uric acid in the blood of gout and nephritis. But from the time of Garrod until the introduction of the Folin method almost no progress was made or even attempted in this field of work.

The recent researches on uric acid in blood have yielded some results of interest in connection with lower animals as well as in man. In order to appreciate these results we must look backward for a moment. A hypothesis to explain gout, based upon the presence of two forms of uric acid in blood, was put forth a number of years ago and the questions involved here have received a great deal of attention from the experimental side ever since. Pfeiffer [26] thought that he had obtained proof of two forms of uric acid in urine, but his work was shown to be erroneous. Kossel and Neumann, Goto,[27] and Minkowski called attention to the fact that uric acid seems to form a compound with thymic acid and Minkowski suggested that uric acid may circulate in the body in this form. There has been no experimental proof that this is the case. One interesting observation of Minkowski's has been interpreted as showing that some purines, at least, are catabolized in the body in combination and not as the free base. Minkowski found that when free adenine was given to dogs the gastro-intestinal tract showed marked inflammation and a crystalline deposit of what he thought was adenine was to be found in the kidneys. These effects did not follow the ingestion of adenine in the form of nucleic acid. Subsequently, Nicolaier [28] showed that the deposits found in the kidneys after adenine ingestion consisted of di-oxy-adenine, thus showing that the organism may oxidize a purine before splitting off the NH_2 group. Such observations as these, and especially the more recent work of Bloch,[29] have tended to show that uric acid may exist in the organism in more than one form, but positive evidence has been lacking.

Through use of the recent methods of blood analysis it has been shown beyond a doubt that in some mammals, at least, uric acid

exists in the blood chiefly in combination. In fresh ox blood, for instance, using the Folin method of determination, we find about one-half of one milligram of uric acid in 100 grams of blood. If, however, the blood filtrate after removal of protein is boiled with hydrochloric acid and then the uric acid determined, it is found that the quantity present is more than ten times that originally obtained. The same figure is ultimately reached if the whole blood is simply allowed to stand, thus indicating that an enzyme is present in blood which can split the uric acid combination. The combined form of uric acid is contained wholly in the corpuscles of the ox blood. In birds, in which uric acid is an end-product, the blood contains none of the combined uric acid and that present is almost wholly in the serum. It is of interest to note that the blood of the ox, an animal which eliminates almost no uric acid in the urine, contains actually about 50 per cent. more uric acid than does that of birds. In the ox blood it is combined and in the bird's blood it is free. These results have been received with considerable skepticism in many quarters, but since the uric acid can be readily quantitatively isolated as such, the correctness of the work is not open to question.[30] The results seem to show that it is probably form rather than quantity of uric acid in blood which is of importance. These findings have been extended to the blood of other species and the results have shown that, with the exception of man, all mammals probably have two forms of uric acid in the blood. In the case of human blood the data so far available are not conclusive. It is quite probable that here, too, uric acid exists in the blood in at least two forms, but they are quite unlike the forms present in ox blood. A new technic is being developed to study this question.

The clinical findings in regard to uric acid in human blood are of considerable interest. The field of work here is new and we must be cautious in drawing conclusions. The recent researches of Folin and his pupils in Boston and of Myers and Fine at the Post-Graduate Medical School in New York have shown that normal human blood contains from one to three milligrams of uric acid in 100 grams of blood. In lead poisoning, in gout, and in nephritis, the uric acid content of the blood is usually

markedly increased, and the determination of uric acid in the blood of suspected gout is of unquestioned value.

In connection with gout the recent researches have shown that the old idea that in this condition the blood becomes "saturated" with uric acid must be abandoned. The solubility of uric acid in blood serum has been shown to be much greater than the concentration of uric acid occurring in the blood of gout. Furthermore, in nephritis the uric acid content of the blood may be quite as high as in gout for long periods of time without any symptom of uric acid deposition occurring. We must therefore assume that in gout there is not only a kidney insufficiency in respect to uric acid elimination, but that there is also a direct vicarious excretion of uric acid from the blood stream into certain tissues where it finally reaches the saturation point and is deposited in the form of sodium acid urate. The view of Minkowski and others that the uric acid circulates in gout in some abormal form, finds some support in the results of studies upon the blood of lower mammals mentioned above. It is my opinion that this view will prove to be the correct one.

In connection with the use of salicylates and of atophan in gout, it is of interest to note that Fine and others have shown that the administration of either of these drugs to gouty patients is followed by a prompt drop in the uric acid content of the blood. Frequently this drop may be so great that the uric acid practically wholly disappears from the blood for a time. With continued administration of either drug, however, the uric acid reaccumulates in the blood. Hence it is of no service to give salicylates or atophan continuously. Whether, by alternating these two drugs for a period of a week or two with each, the blood could be kept relatively free from uric acid continuously, has not yet been determined.

Table VII, for which I am indebted to Professor Myers of the Post-Graduate Hospital, illustrates the findings of Myers and Fine in regard to the early accumulation of uric acid in the blood in nephritis. From this table we should infer that of all the common products of metabolism uric acid is the first to accumulate in the blood when the kidney function is impaired.

Whether uric acid in high concentration in the blood is *per se* toxic to the kidney is not yet known. The frequent development of nephritis in gout might be regarded as lending support to this

TABLE VII

Uric Acid, Urea N. and Creatinine of Blood in Gout and Early and Late Nephritis

Diagnosis	Uric Acid	Urea N	Creatinine	Systolic Blood Pressure
	Mgms. to 100 c.c. Blood			
Typical Cases of Gout	9.5	13	1.1	230
	8.4	12	2.2	164
	7.2	17	2.4	200
	6.8	14	1.7	
Typical Early Interstitial Nephritis	9.5	25	2.5	185
	8.0	37	2.7	150
	5.0	37	3.9	130
	7.1	16	2.0	
	6.6	24	3.3	185
	6.3	18	2.1	
	8.7	20	3.6	100
	7.0	33	2.6	117
	6.3	31	2.1	
	6.3	23	2.4	150
Chronic Diffuse and Chronic Interstitial Nephritis	8.0	80	4.8	240
	4.9	17	2.9	170
	8.3	72	3.2	238
	5.3	21	1.9	145
	9.5	44	3.5	210
	2.5	19	1.9	120
	7.7	67	3.1	
	6.7	17	1.6	165
	8.3	39	2.9	
	6.5	24	3.0	200
Typical Fatal Chronic Interstitial Nephritis	22.4	236	16.7	210
	15.0	240	20.5	225
	14.3	263	22.2	220
	13.0	90	11.1	265
	8.7	144	11.0	225

Myers and Fine: Arch. Int. Med., 1916.

view. Occasionally, apparently normal individuals are encountered whose blood has a uric acid concentration of over 3 milligrams per 100 grams of blood. If such individuals could be followed for some years we would probably obtain valuable data upon the possible etiological importance of uric acid in nephritis.

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MEDICAL EDUCATION IN THE UNITED STATES*

PROF. WILLIAM H. WELCH
Johns Hopkins University

I ASSURE you that it is a great delight to me to have this opportunity—and I seize everyone that presents itself—of returning to my old home. I treasure the associations of those days when I was a part of the profession of New York, and it has been a delight to me to continue in contact with things medical and with my friends and colleagues of this city. I esteem it a high honor to be asked to give one of these lectures. I must pay tribute to the conception underlying the establishment of the Harvey Society. When one considers the purpose of these lectures, the opportunities which they offer, and the influence which they exert, it is an honor for anyone to be asked to be a Harvey Lecturer.

The purpose of the lectures is to present the results of original research. I am rather glad that Dr. Wallace relieved me of the responsibility of having chosen the subject I am to speak on. It would not have been one of my own choice and I question whether it is altogether suitable for this course of lectures. Nevertheless, it is not altogether undesirable that a lecture on medical education should come under this foundation, because everything that concerns research and the conditions favorable for it are dependent upon education, and surely the roots of scientific research lie in the educational system of the country. I think it is more clear than ever in these days, with the establishment of separate research institutions and the interests attaching to scientific investigation in general, that, after all, without a satisfactory foundation on the educational side, research cannot flourish.

It is enough, I think, to point out that such an independent, fruitful research institution as the Rockefeller Institute doubtless would not have justified its establishment twenty-five years

*Delivered April 20, 1916

ago. That is because improvements in medical education had to precede the foundation of such an institution, and I venture to say that themes which relate to all the conditions which affect the development of laboratories, all the material conditions so little understood in general which figure in the development of research, are not out of place in a course of lectures where the prime purpose is to present the results of research.

I am somewhat at a loss how to treat the subject of Medical Education in the United States which has been suggested to me. It is obvious that it is impossible to cover the whole subject and I must ask your indulgence for selecting certain aspects of it, not altogether connected, but such as seem to me to be particularly of primary, or, at least, of special interest.

Nothing is more remarkable in medical conditions in this country than the progress of the last half century in the development of medical education and of medical science, and especially during the latter half of that period. This progress came first in medical education, and as I have already indicated, I think it was a necessary condition for the subsequent development of investigation in medicine. The progress is remarkable when one contrasts it with conditions which had existed before; more remarkable when one contemplates how very far short we still fall of the ideal. We cannot contemplate with any great satisfaction the early history of medical education in America. Probably medical education had nowhere, at any time, fallen to such a low estate as it did during a large part of the last century in our country. The early traditions, which came from Scotland, were sound. They recognized that a medical school should be a part of a university and they also recognized the essential relationship of such a school to a hospital. But with the rapid development of the country, and largely as a consequence of that rapid development, new ideas, essentially novel, unheard of before or since, developed as to the organization of our medical schools. I refer to the establishment of independent medical schools without connection with universities, without vital connection with hospitals, with the power to grant the doctor's degree, and that degree carrying with it the license to practice.

We are so familiar with the existing system in this country that we hardly realize that there has been a distinctive problem in America, the fundamental evil resulting, of course, from the divorce of the medical school from the university and from the hospital, in that each followed its own line of development, with little or no heed to the other. Our problems to-day are, to a very large extent, the result of that condition. They consist to a very large extent in an effort to establish a relationship which should have existed at the beginning between the medical school on the one hand and the university and the hospital on the other. And it is not a little remarkable that on the whole it is much easier to establish the desired relationship with the university than it is with the hospital.

Now I do not wish to be too harsh in judgment of the old order of things in medical education in this country. The system was about as bad as it could be, but there were compensations, and these were undoubtedly due to the character and calibre of the teachers in many instances. Even the characteristic medical schools that are of historic interest in the frontier of America had a very remarkable class of teachers and professors—Nathan Smith, Daniel Drake, McDowell, Dudley. Mere mention of these names to one who knows about the history of medicine in this country is enough to indicate that any young man who came under the influence of such teachers as these must have derived great profit. In other words, the results were better than the system.

I received my own education here in New York before any marked change or improvement had taken place in these conditions, but I entertain and cherish a great feeling of gratitude to many of my teachers. I received stimulus from men like Dalton and Delafield, and later from Dr. Jacobi, my Attending Physician at Bellevue Hospital, and the elder Janeway. Mere mention of these names rouses enthusiasm and interest. We received a stimulus and were brought into contact with high ideals of the profession, notwithstanding all the defects in the system of medical education.

Now a change has taken place, and, as I have indicated, a change so great as to mean a definite break from the old order, and it is worth while inquiring as to some of the factors which are concerned in this improvement. The enumeration of them will enable me to make a few comments of a somewhat general nature.

There has been, for half a century or more, an awakening of professional opinion on the subject, which, however, has had very little effect on medical schools. If one were to enter into a historical review, it would be necessary to go back as far as 1859 when the Medical Department of Northwestern University established a graded course. Later on, at Harvard and at the University of Michigan, improvements came in as regards standards, methods, and certain requirements for admission. But I trust that it will not be deemed immodest if I suggest some of the contributions which the establishment of the Johns Hopkins Medical School in 1893 made to medical education. It had no monopoly of contribution to progress in this direction, but there were certain conditions which enabled us to make rather distinctive advances. In the first place, we were fortunately situated, as things were at that time, on the material side. There existed the Johns Hopkins Hospital and the University, and an endowment, which, although not large, was larger than any existing at that time for the promotion of medical education. There was the standard set by the University in the promotion of a higher university education, as distinguished from college education, so that we realized that we had an opportunity. We felt that it was not worth while to start a new medical school unless we made an addition to the methods of medical education.

When the school was started it had certain preliminary requirements, which still exist, which were not altogether of our own free choice because they were a part of a condition of the endowment which enabled us to begin. I do not propose to discuss in detail the subject of the preliminary education for the study of medicine, but I would point out that the particular requirement which was introduced at that time represented an effort to adjust medical education to the existing, rather anom-

alous condition of general higher education in this country. We require, as you know, a liberal education as represented by a degree in arts and science. Recognizing that the college keeps the students longer than it should for entrance upon professional studies, we ask them to supply training in the sciences fundamental in medicine, Chemistry, Physics, General Biology, with a reading knowledge of French or German. These subjects—Chemistry, Physics, Biology—in the curriculum of European universities come under the medical studies, so that a comparison with these foreign medical schools represents at least a five-year period of study. We ask the college, then, to supply one at least, possibly two or three, subjects which abroad are included in the medical curriculum.

We did not think at the time, nor do we think now, that it is a standard likely to be generally adopted in this country. We have never urged it. It has worked well with us and we are not inclined to make a change. It is an adjustment to existing conditions of higher education. All other efforts to adapt medical education to secondary and collegiate education in America encounter many difficulties. A high school education is not sufficient, unless our high schools develop into something more comparable to the German gymnasium, as there is some tendency to do in the West. But we must try to find a place to stop between the high school and graduation from college. The tendency, which, however, does not seem to be the solution, is to require two years of college work; to bisect transversely, if you will, the college course, and very often associated with that is the telescoping of the last two years of the college course into the professional school, so that two years of professional study are counted both for the Bachelor's degree and for the Doctor's—obviously a makeshift arrangement. The result of this development of the medical school and college or university apart forms a condition which would never have existed if it had not been for the marvelous development on each side. I do not feel, whatever you may mean by a liberal education, that it is highly desirable that it should be demanded by the medical school. Of course the demand that is really desirable, the sort of education which we all feel is so

lacking in most of our medical students, the power of observation, the right attitude toward the subjects he is studying, the capacity to interpret and all of that which is talked of so much now by Mr. Abraham Flexner, may be met by possible improvements in secondary education.

Those of us who are interested in medical education must be very much alive to the possible improvements in secondary education. It is to be hoped that the time will come when the young man may complete his secondary education, have added to that the college education and be enabled to enter upon his medical studies when he is 19 or 20 years of age. This will be solved, I believe, rather by an improvement in secondary education than anything else.

Such, in brief, were our requirements for admission, which still hold, to the medical school. I think we can also point to the organization of the laboratory, or so-called pre-medical subjects, on a more adequate scale than previously existed in this country as a contributing factor in the progress of medical education. The anatomical laboratory, of course, had existed for centuries, from the time of Vesalius, and by virtue of the fact that anatomy was the only subject with which the medical student gained any sort of direct, personal contact with his subject, it had great educational value. It still remains, of course, a fundamental subject, but it has acquired undue prominence in the medical curriculum by virtue of the fact that it was the only subject which was pursued by laboratory methods until recent times. The physiological laboratory is traced, in this country, mainly to the work of Bowditch in Boston and Newell Martin at Johns Hopkins, but it cannot be said, I think, that physiology had taken the place which it should hold in medical education much before a quarter of a century ago. One of the great marks of progress in medical education is due to the recognition of the fundamental nature of physiological study for the training of the physician, so that the study of the activities of the normal body are, to say the least, just as important as a study of the structures of the normal body, and it is a rather distinctive contribution for American medical schools to have established good

laboratory courses for medical students. I see in the audience Dr. Porter of the Harvard School who has had such an influence and done so much in advancing these courses. It is still difficult to arrange an entirely satisfactory routine course for undergraduate students in the physiological laboratory, but we do more in that direction than is done abroad.

The other subjects which we were able to establish upon a fairly adequate basis were pathology, bacteriology, pharmacology and physiological chemistry, and perhaps in the first instance, because this great group of pre-clinical subjects, designated now as laboratory subjects or medical sciences (as if the clinical subjects were not a science), for the first time were adequately organized with laboratories, with a group of teachers as heads of laboratories, with their staff devoting their entire time to the work and with an emphasis upon the practical and laboratory training as compared with didactic lectures or demonstrations of the subjects.

These first two years of the medical course were founded upon certain principles. In the selection of the teachers, they were ever the best to be found or available, but emphasis was made, in that selection, upon the productive capacity of the men. That qualification of the teacher, the productive capacity, is, in a medical school, the important thing and headships were given to men who had earned them by their contributions, and in general their published contributions to their subjects. This guided us at that time.

As regards the clinical side, we at the beginning made slower progress. To Osler, especially, we owe the plan which was adopted. The main thing perhaps was the introduction of the English plan of teaching the fourth year students in the wards of the hospital by the system known as "clinical clerks," a marked advance, I believe, in clinical teaching. The change from the old order was not so striking on the clinical side as on the laboratory side. At once, you might say, the laboratory side of medical education passed from being the weakest, almost nonexistent side, to the strongest side of the medical curriculum.

The plan of the organization of the hospital which was estab-

lished at this time, in 1893, was, I think, a considerable improvement. It consisted mainly in the introduction of a higher professional staff over the internes, so-called house officers; that is, there were resident physicians, surgeons, gynæcologists, obstetricians, over the interne. I have often wondered that this system has not been more widely adopted in this country. It offers a very great advantage. It affords opportunities for the prolonged advanced training of the young men and also the young women who are so fortunate to obtain these positions. The positions are for an indefinite period. The young men devote their entire time, of course, to hospital work and are expected to undertake some investigative work. If you recall the names of those who have held these positions as resident physicians and surgeons, I think you will feel that by the time they have left they have established their reputation, and that the value of that system of organization of the professional staff of the hospital is very clear.

More recently we have come to hope that we shall be able to initiate a very great reform on the clinical side, in the placing of the clinical portion on the university basis by which the heads of the departments may give their entire time to the work. I shall touch on this point later.

These various points, then, I think, mark and set an example for a very considerable improvement in the medical educational system. I do not desire to claim any monopoly on the part of Johns Hopkins University for these advances, because other universities have contributed largely, such as the University of Michigan, but we happened to be first in the field in many of these directions, and I think the plan adopted by Johns Hopkins is one factor which has advanced medical education in this country.

The State Licensing Boards have had great influence in exerting pressure on the inferior medical schools, crowding them to the wall and very often driving them out of existence. The principle, of course, is that the license to practice should go with the granting of the degree of Doctor of Medicine, especially when one considers the system and the conditions under which the degree is granted. The influence of these State Licensing Boards

has thus been very good in bringing up the general average. They have been of no particular assistance and some time ago almost threatened to be a handicap to the better medical schools. Of course we all recognize what such examinations should be. The character of these examinations falls very far short of the ideal. especially in the lack—although there is an improvement with time—but in general, in the lack of a practical examination, so that it is not any real test of the power of the student to use the implements of his profession or of his real living knowledge of the subject. They will improve, doubtless, and it is to be expected that in time conditions will be such that those on the Examining Board will be also teachers in our schools.

The Council on Medical Education of the American Medical Association and the Association of American Medical Colleges have done a great deal in improving conditions, especially in leading professional opinion on the subject and inciting to a very considerable degree a moral pressure. There have been at times, I am frank to say, certain tendencies in the Council on Medical Education to make one pause. I refer to the efforts to "standardize the curriculum." I think it a very horrible thing to attempt to indicate the number of hours, for example, to be devoted to the study of a subject, and at one time our State Licensing Boards seemed inclined to introduce some such scheme. Of course we want as elastic a condition as possible. When one considers the importance of adjusting medical education to the changes and advancing conditions of medical knowledge, how absurd to attempt to specify the number of hours to be given to any subject, bacteriology for example. Only a few years ago the subjects of immunology and serology were not thought of as belonging in the medical curriculum, but to-day things have changed and they should be a very important part of the medical curriculum. We owe our great working policy in medical education to the conferences held annually in Chicago, attended by leading educators, not only in medicine but other subjects as well. Such conferences are very valuable and the publications very interesting and often important.

Another great factor is Dr. Abraham Flexner's report for

the Carnegie Foundation. I consider it to be one of the most remarkable and influential publications in educational literature. It has had not only a large influence upon the professional opinion, but especially a large influence on universities and upon public opinion. It is to be characterized as one of the important factors which illustrate this remarkable advance in medical education.

But of course the progress of medicine lies back of it all. The face of medicine has changed greatly in the last thirty or forty years, although it is the same medicine in many ways. That medical education should continue without advance during all the great discoveries characteristic of this era, would hardly be conceivable.

I have run briefly over the history of some of these factors, because I wish to make some comments of a more general character. I have already spoken of the development of the laboratory subjects. It is worth repeating, perhaps, that it was a consequence of the organization of the laboratories of anatomy, physiology, pharmacology, bacteriology, etc., and the selection of men devoting their entire time to the work, selected on the basis of scientific ability, that these great sciences have progressed to the point which they have in this country and of which we are so proud. To give an instance of the close relationship between the progress of medical sciences on the one hand, and of our educational system on the other, it was only two or three years after our medical school had opened that we started the *Journal of Experimental Medicine.* It was the pioneer journal devoted to the publication of papers of a more or less technical or monographic character in these sciences. I recall so well the doubt expressed as to whether there existed enough material of the sort which was desired to keep the journal alive. We never dreamed of limiting it to any one of these so-called laboratory subjects. We endeavored to select a title which excluded merely practical, clinical medicine, and was not restricted to any one line of research. I cite all this as an example of conditions which existed only a short time ago. It was within two or three years that Dr. Porter found the time had come to establish a *Journal of Physiology,* which was the first offshoot from the

Journal of Experimental Medicine, and then came in rapid succession, the *Journal of Anatomy, Journal of Biological Chemistry, Journal of Medical Research, Journal of Infectious Diseases, Journal of Pharmacology and Therapeutics,* and still more recently the *Journal of Bacteriology* and the *Journal of Immunology.* Is it not wonderful that in a comparatively short space of time these subjects should have developed to the height of which we are so proud?

America to-day, as a contributor to the various sciences of medicine, stands in a position to medicine commensurate with the size and importance of the country. We lay, I believe, probably greater emphasis upon the teaching of undergraduate medical students in the laboratory than is done elsewhere; we devote more time to the teaching of undergraduate medical subjects by laboratory courses in certain subjects particularly—I have already cited them—than is done abroad. There are already developed certain distinctive characteristics of our American medical schools, and this is one of them. Of course it makes us inquire whether we are possibly giving undue prominence to some subjects, but I would be the last one to admit that, although at the same time we should bear in mind certain things. We cannot teach in the laboratory more than a very small fraction of the contents of the subject; only a part of it, and that not necessarily the most significant and important. In other words, is there not some risk of acquiring too restricted and limited a conception? Is there not some risk of a loss of perspective in the subject by exclusive emphasis upon teaching in the laboratory? I believe so firmly in the laboratory method in imparting that kind of knowledge which is really vital, a knowledge that gives power, that I do not wish to be misunderstood and be thought to minimize its value, but I think we must supplement the laboratory teaching by efforts to secure these broader conceptions and this clearer perspective. I have never been willing to give up altogether the lecture. If one does not believe in lecturing, I think he had better not lecture. I think there is some value in a lecture, and I think proper emphasis in lectures and recitations will enable teachers to

stimulate the student and exert some pressure to make him read. The students do not read enough. As a rule they know only the subjects which are taught in the laboratory. I will not labor the point, but I would emphasize the fact that we should consider it very carefully.

I turn now from the laboratory side of medical education to the clinical side. That, of course, is the central feature. The teaching of the clinical subjects should be carried out along the same general lines. At the start there were efforts in this direction, especially in the use of students in the wards of the hospital, acting as clinical clerks and surgical dressers. I shall not attempt to discuss this system. The plan of organization of the professional staff shall always remain a controversy between the clinical and laboratory side.

When one considers what should be the functions of the head of a principal department of medicine, when one considers that he is responsible for the teaching, responsible for stimulating investigation and for having the right sort of men for the conduct of investigation in his field, responsible for the study and care of the patients in the hospital, and the whole organization of the department, it sems to me that it requires no argument that whoever assumes that responsible position as head of a clinical department should be prepared to devote his entire time to it. There is no time to engage in an outside practice. I know that it is urged that the clinical teacher who limits his experience to patients in the hospital is deprived of a very valuable experience to be derived from outside practice. It is a valuable experience undoubtedly. I think it would be more valuable if he had a rural practice. I doubt if anything in the ordinary conditions of a consulting practice in the city is as likely to develop resourcefulness in a physician as a rural practice. In a word, of course, the more varied the experience of the clinician is to be, the more must he be brought in contact with patients and unusual conditions, but there are limits to human endurance, time and energy, and the question is, what is the best use of his time? Can we doubt whether it can be successfully maintained that the expenditure of time in seeing

patients in consulting practice is as valuable to him as the study of cases of diseases in the hospital under all of the opportunities which exist there? The time has gone by when a man can do both competently and with justice to his position as the head of an important clinical department in the medical school.

How this condition is to be brought about is, of course, very important. We endeavored at Johns Hopkins University to do this by making no compromises. Through a generous appropriation from the General Education Board, we have been enabled to place three of our main clinical departments, those of medicine, surgery and pediatrics, upon the so-called " university basis, " or, as more commonly called, the " full-time " system. I do not particularly like the name; for teachers under this system are the only ones who have any leisure time.

Of course the heads of departments should not be prohibited under the new arrangement from seeing private patients, but they are paid such salaries through this endowment that there is no necessity for them to earn a livelihood through private patients. They can see them if they like, but not having any financial difficulties, they will see only those that are of special interest to them. Now, our experience thus far shows that the amount of this private practice is kept within pretty narrow limits by the withdrawal of financial necessity. The patient, of course, pays a fee, but the fee goes to the fund for the promotion of the system. I do not see very well how one could justify the raising of a large sum for clinical heads on the university basis if they should supplement their income from private practice. This would be a great injustice to the laboratory men. The salaries which they receive are much larger than those received by the laboratory men. I do not think outside salary limits desirable for university professors, at least I think university professors who are of the calibre of the men occupying these positions ought to receive similar salaries, but, as a matter of fact, I think you can justify a somewhat larger salary to the heads of clinical departments on the ground that they are serving the hospital as well as the university; that they have very responsible duties in the care of the patients and that after

all a clinical department, with its staff and hospital branches of clinical and investigatory laboratories, is a larger undertaking than a single laboratory, so that one can defend the paying of larger salaries. But it is sufficient, I think, to say that the opportunity has been presented to us and we have been glad to initiate this system and to pay these salaries. How widely the system as we have adopted it should be generally applied, I am unable to say. It has no saving virtue in itself; it is the men who operate it who are fully responsible for its failure or success. To introduce it where conditions are not suitable, where the hospital does not afford the requisite patients and laboratory facilities, and the staff of full-time young men, would be useless. It is only where conditions are suitable that the system should be adopted, but when it is carried out in the uncompromising way that we have done, it undoubtedly marks, I think, one of the greatest improvements in medical education of recent times, and is bound to exert a very great influence on the character of organization of the medical school. We have had it for two years and we like it. I think it has passed the experimental stage as far as we are concerned. I do not wish to say that we are satisfied with our conditions, but it gave us the opportunity to make a very great improvement and we were glad to seize that opportunity. The plan does not necessarily do away with the services of part-time men in the school. Whatever faults there may be in this condition, the outside work is very valuable for a man and makes him a better teacher. He finds a place in the school, only he is no longer the head of the department. I take it that this marks a new career for young men. The very fact that what seems to so many a serious objection in a curriculum, the difficulty of filling positions with men who are qualified for this kind of work, is in itself something of a criticism of the existing system and I believe that one of the great dangers of the new order of things would be the opening up of a most attractive career leading to that of consulting physician. But I do not know what could be better than to enter into such an opportunity as is now offered, devoting one's time to the study of problems of disease as they are presented by the living tissue, to work in the laboratory and study at the bedside.

As regards the establishment of the proper relations of the hospital to the medical school, there is much that can be said, but the time has gone by when it is necessary any longer to emphasize the great service which the hospital devoted to education and scientific work, as well as the humane care of the patients, does for the community. But it is necessary to dwell on the character of organization of the university clinic, as distinguished from the general hospital. You know, those who are familiar with medical education abroad, especially in Germany, that part of it is the clinic, the rest a general hospital. In other words, the mere saying on the part of the trustees that you can use the hospital, is not enough. It is a very considerable undertaking to transform it in whole or in part into a general university clinic, meaning by that that there is one man in charge with a staff of men, assistants or associates, with a chemical and biological laboratory available for the study and investigation of problems of disease, and all the necessary arrangements for teaching and the treatment of patients grouped as a single department. That is, in a word, what I conceive to be the proper organization of the true university clinic. I understand that efforts are being made here in New York to establish a clinic on that basis and everyone must realize how important it is to have the right conception of what a true university clinic should be.

I have jotted down a great many things, but let me just give them in a word. Certain of these other topics are of especial interest to me. I would like to say something on the general subject of research and teaching and also on the relationship of the independent research institution to educational institutions in general. There is a little apprehension, particularly on the part of the university, that the independent research institutions, like the Carnegie Institute of Washington and the Rockefeller Institute, are getting too much attention; that they draw the able investigator from educational institutions; that they tend to create dissatisfaction. I think, on the other hand, that these research institutions have abundantly justified their existence by their contributions to science. That is, indeed, quite

obvious. But I think as time goes on that they will supplement the educational institutions. Anything that increases the opportunities and rewards for the scientific worker is undoubtedly of very great advantage. One reason why Germany has obtained such a high stage in scientific investigation has been because the career of the scientific man was made attractive. By rewards I do not mean so much the pecuniary ones as the satisfaction which comes from contributions, the esteem in which the worker is held by the community. I think the opportunity for these careers in this country are enormous and rendered more attractive by the establishment of these institutions. It is true, of course, that some of the very best trained men are withdrawn from the educational field by their work in the research institutions, but it is of very great advantage to the teaching institution to know that such positions are available for students. It increases their value, I think, in that way very much. It acts as a stimulus on the educational institution to further research. As time goes on there will begin to return to the educational institutions men who have had this very superior training in research. I believe, on the whole, that over-multiplication would be unsatisfactory. The future relation between the independent research institution and the educational institution—of course we are speaking of the medical school and medical research institution—the mutual relations, will be advantageous and each is going to be of great help in the end to the other.

I must omit a great many topics, which I should like to have touched upon. I wanted to say something about the medical curriculum, optional courses, and many other things.

I do not wish to leave the impression that there are no great deficiencies in our own medical school. I have enlarged upon the progress which has been made more in contrast with the past than from a feeling that we have begun to approach the goal. It would be interesting to point out and to dwell upon some of the deficiencies, but time allows an enumeration of just a few.

We are lacking in the proper cultivation of legal medicine, a very important subject and one of importance to the clinician.

Of course we all recognize that one of the great needs of medical education is the establishment of institutions of hygiene. I would like to have said a few words about the teaching of the history of medicine in our medical schools. It adds greatly to the attractiveness of medical study, and I believe also to the enjoyment of the physician later in his professional work, to find how knowledge came to be. I do not advocate systematic lectures on this subject. I do not know of anything that would be more definitely dull and uninteresting, but there are other ways of cultivating this subject.

Are we training men to serve the community in the treatment of disease any better than they did in the old days? From the beginning the aim in medical training has been to enable the physician to prevent and cure disease and injury, to relieve suffering, and to preserve health. These aims are the same to-day as they were forty years ago. It is this consistency of purpose which gives the wonderful interest and continuity to the study of medicine. Notwithstanding all of the wanderings of the past, we are striving for the same aim as before. There have been opened out new fields, new vistas, new methods, so that what was suitable for a training to meet these great aims in the past is no longer the best available. The fundamental thing, the fundamental problem in medicine, is to train men to use the resources of the medical science and art most efficiently for the prevention and cure of disease, and I believe that while many of the commonest ailments of mankind are no better treated to-day than in former days, we are acquiring a new kind of knowledge of disease more important in its practical values. We feel that the existing knowledge and resources of the medical art are only imperfectly realized, and my belief is that the newer methods of medical education can be most useful in enabling the student to acquire a better scientific knowledge of the nature of disease and enabling him to apply this knowledge more successfully in the treatment and prevention of disease.